NASA-TM-108029

P.133

LIFE SCIENCES FLIGHT EXPERIMENTS PROGRAM

SPACELAB-4 SCIENCE SUMMARIES OF TENTATIVELY SELECTED EXPERIMENTS

(NASA-TM-108029) LIFE SCIENCES FLIGHT EXPERIMENTS PROGRAM. SPACELAB-4 SCIENCE SUMMARIES OF TENTATIVELY SELECTED EXPERIMENTS (NASA) 133 p

Unclas

29/91 0132840

SCIENTIFIC OBJECTIVES AND FEATURES

OF THE FIRST DEDICATED LIFE SCIENCES MISSION -- SPACELAB-4

- 25 separate investigations combined into a comprehensive, integrated exploration of the effects of acute weightlessness on living systems
- Emphasis on known problems of manned space flight
- Complementary designs in human and animal investigations to validate animal models for human physiology in weightlessness
- Experimental subjects include humans, squirrel monkeys, laboratory rats, plants, and frogs
- Primary scientific objectives include the study of:
  - Acute fluid shift
  - Cardiovascular adaptation to weightlessness
  - Vestibular physiology including space sickness
- Secondary scientific objectives include the study of:
  - Red cell mass reduction
  - Negative nitrogen balance
  - Negative calcium balance
  - Suppressed lymphocyte reactivity
  - Gravitropism and phototropism
  - Fertilization and early development of frog eggs

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THE FIRST LIFE SCIENCES DEDICATED MISSION -- SPACELAB-4

This brochure has been prepared from official NASA documents to give a reader with a comprehensive life and biomedical sciences background specific but abbreviated information relative to the twenty-five experiments which have been tentatively selected for the first dedicated life sciences mission, Spacelab-4 (SL-4), planned for launch in December 1985. It is vitally important for the reader to understand that these 25 experiments were selected on a stand-alone basis but that during the "development for flight phase" of SL-4 (which continues at this writing, November 1982), they are being coordinated into a unitized life sciences "mission" which, while retaining the individual experiment's objectives, will share data, specimens, equipment and the Spacelab crew. SL-4 is, therefore, the most ambitious interdisciplinary life sciences flight mission, using the most sophisticated equipment and scientific protocols, ever undertaken by the Space Agency. A brief chronology will give an overview of the mission's maturation to this point:

1978 Dissemination of Announcement of Opportunity-OSS-1-78 to the scientific community

1978 Receipt of 373 proposals in response to above "AO"

1978 Peer review of above proposals by outside (AIBS) and NASA peer review committees

1979 "Definition phase" of SL-4 mission began with 117 experiments

1981 Tentative selection of 25 of the 117 defined experiments -- "Development for flight" phase initiated

The 25 investigations are arranged in eight discipline groups which are divided by light blue separation pages. An acronym list is included to help the reader with definitions of NASA-specific abbreviations. Inside the front cover is a synopsized philosophy of the guidelines which were used in formulating this life sciences Spacelab mission.

ACRONYM LIST

ACTH	ADRENOCORTIOCOTROPHIC HORMONE
AFT	AUTOGENIC FEEDBACK TRAINING
AO	ANNOUNCEMENT OF OPPORTUNITY, OSS AO-1-78
ARC	NASA AMES RESEARCH CENTER, MOFFETT FIELD, CA
ASTP	APOLLO-SOYUZ TEST PROJECT
BMR	BASAL METABOLIC RATE
BP	BLOOD PRESSURE (ARTERIAL)
BTS	BIOTELEMETRY SYSTEM
CDMS	COMMAND DATA MONITORING SYSTEM
CO	CARDIAC OUTPUT
CSST	CORIOLIS SICKNESS SUSCEPTIBILITY TEST
CT	COMPUTERIZED TOMOGRAPHY
CVP	CENTRAL VENOUS PRESSURE
D-1	"DEUTSCHLAND ONE"/THE FIRST GERMAN DEDICATED SPACELAB
DEMS	DYNAMIC ENVIRONMENT MONITORING SYSTEM
DEO	DEVELOPMENT, ENGINEERING AND OPERATIONS CONTRACTOR/MATSCO
DOPA	DOPAMINE
DNA	DEOXYRIBONUCLEIC ACID
E	EPINEPHRINE (ALSO ADRENALIN)
ECG	ELECTROCARDIOGRAM/SOMETIMES "EKG"
EMG	ELECTROMYOGRAM
EMP	EXPERIMENT MANAGEMENT PLAN
ENG	ELECTRONYSTAGMOGRAM
EOG	ELECTROOCULOGRAM
EP	ERYTHROPOIETIN
ERD	EXPERIMENT REQUIREMENTS DOCUMENT
ESA	EUROPEAN SPACE AGENCY
FO	FUNCTIONAL OBJECTIVE
FOP	FLIGHT OSCILLATING PLATFORM
FSH	FOLLICLE STIMULATING HORMONE
GABA	GAMMA-AMINO BUTYRIC ACID
GFE	GOVERMENT FURNISHED EQUIPMENT
GH	GROWTH HORMONE
GPWS	GENERAL PURPOSE WORK STATION
GSE	GROUND SUPPORT EQUIPMENT
GTT	GLUCOSE TOLERANCE TEST
1-G	NORMAL FORCE OF EARTH'S GRAVITATIONAL FIELD/ONE-G
0-G	NULL GRAVITY/HYPOGRAVITY/AS EXPERIENCED IN ORBITAL SPACEFLIGHT
HQ	NASA HEADQUARTERS, WASHINGTON, DC
HR	HEART RATE, SOMETIMES "PULSE RATE"
IR	INFRARED
IWG	INVESTIGATOR WORKING GROUP

ACRONYM LIST

JSC	NASA JOHNSON SPACE CENTER, HOUSTON, TX
KSC	NASA KENNEDY SPACE CENTER, KENNEDY SPACE CENTER, FL
L	"LAUNCH" (IF - VALUE, PREFLIGHT; IF + VALUE, INFLIGHT)
LBNP	LOWER BODY NEGATIVE PRESSURE DEVICE/TANK
L:D	LIGHT TO DARK CYCLE IN HOURS
LH	LUTEINIZING HORMONE
CMMD	LARGE MASS MEASUREMENT DEVICE
LS-1	"LIFE SCIENCES ONE"/SL-4/THE FIRST U.S. DEDICATED LIFE SCIENCES SPACELAB MISSION
LSAC	LIFE SCIENCE ADVISORY COMMITTEE
LSFEP	LIFE SCIENCE FLIGHT EXPERIMENTS PROGRAM
LSLE	LIFE SCIENCE LABORATORY EQUIPMENT
LSSC	LIFE SCIENCE SUBCOMMITTEE TO THE SPACE SCIENCES STERRING COMMITTEE
MAMA	MULTIPLE ANIMAL METABOLIC APPARATUS
MAO	MONOAMINE OXIDASE
MD	MISSION DAY
MT	MAGNETIC TAPE
NE	NOREPINEPHRINE/NORADRENALIN
OFO	ORBITING FROG OTOLITH EXPERIMENT
OSS	OFFICE OF SPACE SCIENCE, NASA HEADQUARTERS
OTT	ORTHOSTATIC TOLERANCE TEST
OVR	OFF-VERTICAL ROTATION
PGU	PLANT GROWTH UNIT
PHA	PHYTOHEMAGGLUTININ
PI	PRINCIPAL INVESTIGATOR
PMHU	POCKET MOUSE HOUSING UNIT
PMN	POLYMORPHONUCLEAR NEUTROPHILS
PMS	PHYSIOLOGICAL MONITORING SYSTEM
POP	PROGRAM OPERATING PLAN
PPB	PAYLOAD POLICY BOARD
PU	PANEL UNIT
R	"RECOVERY"/DAYS AFTER LANDING OF SPACE SHUTTLE/A + VALUE
RAHF	RESEARCH ANIMAL HOLDING FACILITY
RAU	REMOTE ACQUISITION UNIT
RBC	RED BLOOD CELL
RNA	RIBONUCLEIC ACID
RSBCU	RODENT SACRIFICE/BLOOD COLLECTION UNIT
SCR	STRIP CHART RECORDER
SEM/TEM	SCANNING ELECTRON MICROSCOPY/TRANSMISSION ELECTRON MICROSCOPY
SLED	ESA VESTIBULAR LINEAR ACCELERATOR
SL-1,2,3,&4	SPACELAB FLIGHTS #1, #2, #3, #4, (#4 SCHEDULED TO BE LS-1)

ACRONYM LIST

SMA	SCIENCE MONITORING AREA AT JSC
SR&T	SUPPORTING RESEARCH AND TECHNOLOGY
SRS	SUBJECT RESTRAINT SYSTEM
SSSC	SPACE SCIENCE STEERING COMMITTEE/NASA HEADQUARTERS
SST	SUDDEN STOP TEST
SWT	SCIENCE WORKING TEAM
TBD	TO BE DETERMINED
TSH	THYROID STIMULATING HORMONE
ULMS	ULTRASONIC LIMB MEASURING SYSTEM
UMS	URINE MONITORING SYSTEM
VOR	VESTIBULO-OCULAR REFLEX
WBOA	WHOLE BODY OSCILLATING ACCELERATION

CARDIOVASCULAR/CARDIOPULMONARY

PROPOSAL NUMBER: 781294

TITLE: Cardiovascular Adaptation to Zero Gravity

SPECIMEN: Human

PRINCIPAL INVESTIGATOR: Blomqvist, C. G., M.D.
AFFILIATION: University of Texas at Dallas, TX

CoIs/AFFILIATIONS:
J. H. Mitchell, M.D./University of Texas at Dallas, TX
F. A. Gaffney, M.D./University of Texas at Dallas, TX

BACKGROUND: Dr. Blomqvist plans to demonstrate that the human cardiovascular system undergoes rapid and effective adaptation to 0-g, that major cardiovascular control mechanisms remain intact, and that postflight cardiovascular "deconditioning" (at least on 7-day missions) is merely a functional hypovolemia. Dr. Blomqvist also hopes to establish 24-hour, 5-degree head-down bed rest as a model for studies of acute cardiovascular response to weightlessness.

Dr. Blomqvist wanted to establish the efficacy of body surface cooling as a countermeasure for orthostatic intolerance encountered during readaptation to 1-g. NASA has elected not to pursue this objective.

The experiment includes invasive measurement of central venous pressure from launch until 8-12 hours into the mission. The major fluid-volume portion of the experiment includes estimation of cardiac output, heart rate, indirect arterial pressure, forearm venous compliance, 2-D echocardiography, blood volume, and key plasma hormone levels. Measurements would be made at rest, during passive cardiovascular stress, lower body negative pressure (LBNP), and during active cardiovascular stress (cycle ergometry).

This experiment is a comprehensive, extension of the Skylab cardiovascular studies. The studies are responsive to Announcement of Opportunity (AO) criteria to extend prior observations on human subjects during adaptation to space flight.

This experiment is a key element in the design of the LS-1 mission. The proposal forms a framework for careful integration of fluid-electrolyte and cardiovascular studies.

PI OBJECTIVES: To investigate in detail the cardiovascular adaptation to 0-g, including the overall circulatory and hormonal responses to the headward fluid shift and its impact on cardiovascular control mechanisms. To test the validity of 24-hour head-down tilt as a model of 0-g by comparing data obtained in pre-flight simulation studies and during actual flight in the same group of crew members. To obtain preliminary information on the value of body surface cooling as a short-term countermeasure against cardiovascular deconditioning.

PI HYPOTHESES: Cardiovascular adaptation to 0-g is rapid and effective and consists primarily of a systemic response to an early head-ward shift of body fluids. The degree of cardiovascular dysfunction in space is minor and the

major regulatory mechanisms remain intact. Post-flight cardiovascular dysfunction, which includes functional hypovolemia, is a direct result of an effective adaptation to 0-g that suddenly has been rendered inappropriate by the return to normal gravity. Bed rest with head-down tilt provides a satisfactory model of 0-g for studies of cardiovascular function. The essential features of the cardiovascular adaptation to 0-g can be reproduced during a 24-hour period of bed rest with head-down tilt at 5°. Body surface cooling is an effective, safe, and physiologically appropriate countermeasure against cardiovascular dysfunction during re-entry.

EXPERIMENT PLAN:

Preflight

1. Early preflight studies

Several measurements will be obtained in all four subjects at L*-180±30 days and repeated at L-90±15 days. The set includes: (a) maximal exercise tests in the upright and supine position with measurements of cardiac output *L - Launch and respiratory gas analysis, (b) LBNP with measurements of cardiac output and forearm flow, (c) pharmacological evaluation of autonomic cardiovascular regulation, (d) 2-dimensional echocardiographic studies, and (e) characterization of body dimensions and fluid compartments.

2. A comprehensive study of the response to 0-g simulated by a 24-hour period of bed rest with head-down tilt at 5°.

This study will be performed at L-30 to -60 days in two subjects (Payload Specialists).

3. Late preflight studies

The measurement set summarized under (1) will be repeated at L-7 to L-10 days.

4. Immediate preflight studies

These studies will be performed within 12 hours of launch and include insertion of a central venous catheter for pressure measurement (CVP) in two Payload Specialist subjects, 2-d echocardiogrpahy, cardiac output (supine, sitting and standing) plasma hormone levels, body weight, and blood volume.

Inflight

Central venous pressure, indirect arterial pressure and heart rate will be monitored in two subjects (Payload Specialists) starting as soon as possible after launch and continuing through L+8 hours at which time the CVP lines will be removed.

All four subjects will be studied at L+6 to 10 hours with measurements at rest of cardiac output, heart rate, indirect arterial pressure, forearm venous compliance, 2-d echocardiography, blood volume (as estimated from hematocrit), plasma hormone levels, body weight, and static leg volume.

L+24 to 36 hours. All four subjects will be studied with LBNP, including measurements of cardiac output and forearm flow, a maximal exercise test, also with cardiac output measurements and respiratory gas analysis. Body weight, leg volume, plasma hormones, blood volume by hematocrit will also measured.

Day L+2 through R-3. Daily measurements of cardiac output, heart rate, indirect arterial pressure, 2-d echocardiography, and body weight will be performed.

Day R-2 and R-1. The major inflight studies which will be performed at this time include measurement of plasma hormones (samples will be collected), body weight, LBNP, a maximal exercise test, pharmacological autonomic testing, and 2-d echocardiography. All four subjects will be included.

Day R-0 (L+7 or L+10). Measurements of cardiac output, heart rate, indirect arterial pressure, body weight and 2-d echocardiography will be performed.

Postflight

Day R+0. Central venous catheters are reinserted within R+6 hours in the two Payload Specialists subjects and will remain for 24 hours. Cardiac output, heart rate, and indirect arterial pressure are measured in all four subjects at rest, supine, sitting and standing. 2-d echocardiography is also performed within R+12 hours. Blood samples will be drawn for measurement of plasma hormones. Blood volume, body weight, and leg volume will also measured.

Day R+1, R+7 to 10, and R+40 to 50. The complete set of cardiovascular studies performed at R-2 and R-1 will be repeated in all four subjects.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The definition phase output has clarified areas in the original proposal related to protocol, crew requirements, and flight hardware requirements. The number of subjects required has increased from three to five. All inflight studies will be performed on five crewmen (three Payload Specialists and two Mission Specialists) except that central venous pressure measurements using catheters will be performed on the three PS's only. The catheters can now be removed 8 hours afer launch rather than 12 hours as originally proposed. The requirement that the three investigators be considered as crewmen due to complexity of the experiment has been removed. One of the crewmen should be an M.D. with clinical experience and appropriate training and another should be an M.D. or life sciences Ph.D. with suitable experience. The original concern over the use of a CVP catheter is now eliminated by improved techniques and greater experience.

SCIENCE: Dr. Blomqvist's investigation will yield significant new information concerning human cardiovascular adaptation to weightlessness. The experimental design involves the broadest array of proposed cardiovascular measurements before, during, and after weightless exposure. These measurements involve many familiar clinical procedures that are performed daily in laboratories across the nation. The use of such procedures permits a confident estimate of the likelihood of success in space and, indeed, it is high.

The proposal directly addresses a known problem of manned spaceflight which constitutes the first AO evaluation criterion. It is also quite consistent with both payload and program objectives.

This proposal involves the use of an indwelling catheter to measure central venous pressure. At the time of categorization there were technical and safety concerns over the use of such a catheter aloft and, for these reasons, the proposal was assigned category 3. These concerns were carefully explored during definition, and we are now confident that this important measurement is technically feasible in Spacelab and can be performed in such a manner as to pose no significant risk to the subject.

EQUIPMENT: This experiment involves some 30 pieces of flight equipment. Most devices are small and already in the LSLE inventory. Major devices include two microcomputers, a physiological signal conditioning system, a mass spectrometer, a two dimensional echocardiograph, a bicycle ergometer, a lower body negative pressure device, and a venous pressure measuring system. All but the last of these will come from the LSLE inventory. The last device is a minor system already developed by the PI. Several devices not already in the LSLE inventory will be acquired by JSC through competitive procurement.

SUMMARY: This is an experiment which has undergone significant development during the definition phase. The questions related to the original categorization have been resolved in that the experiment can now be successfully completed. Catheter placement has been evaluated and is no longer viewed as a problem. Comprehensive ground-based studies have also been completed and no problems were discovered. Blomqvist's experiment represents the most comprehensive cardiovascular experiment proposed. This experiment directly addresses the first AO evaluation criterion and is consistent with program objectives. It is recommended that this experiment tentatively be selected for flight with the following modifications: Dr. Farhi will do the specified cardiac output and related cardiopulmonary measurements with his method and the functional objective to establish the efficacy of body surface cooling as a countermeasure will be deleted.

FLOW CHART AND MEASUREMENT TABLE FOR EXPERIMENT #294 - BLOMQVIST

TESTS	Preflight					Launch	Inflight								Recovery	Postflight				
	L -180	L -90	L -45*	L -7	L -0		L +2H	L +8H	L +1D	L +2	L +3	L +4	L +5	L +6		R +6H	R +12H	R +1D	R +7	R +45
BICYCLE EXERCISE TEST: ECG, HR, Indirect BP (cuff) C.O. (C_2H_2-rebreathing technique) O_2 uptake, CO_2 production, tidal volume, respiratory rate	4	4	2* (post)	4					4			4	4					4	4	4
POSTURAL CARDIOVASCULAR RESPONSE: C.O. - (C_2H_2-rebreathing), HR, indirect BP (cuff)			2* (pre)		2												4	4	4	4
BODY WEIGHT/MASS AND STATIC LEG VOLUME (multiple circumference)	4	4	2*	4	2			4	4	4	4	4	4	4		4	4	4	4	4
BLOOD VOLUME: RCM-(Cr-51 method) PV-(RISA technique on days L-7 and R+0; otherwise, Evans blue-hematocrit method			2*	4	2			4	4								4			
CENTRAL VENOUS PRESSURE-INVASIVE: HR, indirect BP (cuff)			2*		2		2	2								2	2			
24-HOUR HEAD-DOWN TILT AT 5°: Performed on day L-45 Only, See * items			2*																	
BASIC CARDIOVASCULAR MEASUREMENTS†: C.O. C_2H_2 rebreathing), lung tissue volume, pulmonary diffusing capacity, ECG, H.R., indirect BP (cuff)								4		4	4			4						
FOREARM VENOUS COMPLIANCE: (using occlusion plethysmography)			2*					4									4	4	4	4
ECHOCARDIOGRAPHY (2-dimensional):	4	4	2*	4	2			4	4	4	4	4	4	4			4	4	4	4
PLASMA HORMONES/HEMATOCRIT: radioimmunoassay of renin, aldosterone, ADH, epinephrine, norepinephrine	4	4	2*	4	2			4	4			4	4				4	4	4	4
LBNP - (standard Skylab protocol): ECG, HR, leg volume, indirect BP (cuff), C.O., forearm flow (occlusion plethysmography)	4	4	2* pre/ post	4					4			4	4					4	4	4
AUTONOMIC FUNCTION TEST OF β and α - ADRENERGIC STIMULATION ECG, HR, indirect BP (cuff), C.O., forearm flow, plasma hormone levels	4	4	2* pre/ post	4								4	4					4	4	4

† These measurements will be obtained preflight and postflight during the other protocols and tests.

* 24-hour head-down tilt test

PROPOSAL NUMBER: 781066

TITLE: In-flight Study of Cardiovascular Deconditioning

SPECIMEN: Human

PRINCIPAL INVESTIGATOR: Farhi, L. E., M.D.
AFFILIATION: State University of New York at Buffalo, NY

CoIs/AFFILIATIONS:
C. F. Sawin, Ph.D./Perkin-Elmer Corporation, Pomona, CA
A. J. Olszowka, M.D./State University of New York at Buffalo, NY
R. A. Morin/State University of New York at Buffalo, NY

BACKGROUND: Dr. Farhi's plan is to quantify cardiopulmonary deconditioning that occurs during acute exposure to weightlessness. The proposed effort is an extension of Dr. Farhi's prior ground based studies of physiological effects of gravity.

The technical approach is based on a non-invasive rebreathing technique for measuring a number of cardiovascular and respiratory variables, such as cardiac output, stroke volume, functional residual capacity, lung tissue volume, effects of maldistribution of ventilation and perfusion, and estimates of mean tissue oxygen and carbon dioxide tensions. These values would be determined during rest and mild cycle ergometer exercise and before, during, and after a space mission.

This novel technique for the estimation of cardiac output requires no special tracer gas (e.g., nitrous oxide, acetylene), requires minimal training, and imposes no stress on subjects. The short time required for a complete set of measurements permits the investigator to make several determinations in one experimental condition; this minimizes the effects of momentary variations and of analytical errors. The absence of simple, reliable, non-invasive methods for the estimation of cardiac output in a space vehicle has limited prior cardiac output data to pre- and postflight measurements (non-invasive).

Dr. Farhi recognizes that the study of cardiovascular adjustments in weightlessness must be based on measurements of several variables in addition to cardiac output. This understanding supports the NASA recommendation to combine Dr. Farhi's proposal with the more comprehensive proposal of Dr. Blomqvist.

The specific contribution of this proposal is the reliable, frequent, operationally safe assessment of cardiac output using non-invasive techniques.

PI OBJECTIVES: To assess the immediate cardiovascular and respiratory readjustments that occur when a subject is exposed to 0-g. To determine whether prolonged exposure to 0-g results in delayed changes in cardiovascular variables; to measure the direction and extent of such changes. To determine how acute and prolonged exposures to 0-g affect the subject's response to increased physical activity. To determine the time course of readjustments in a subject previously exposed to 0-g and returned to normal gravity.

PI HYPOTHESES: Acute exposure to 0-g affects the following variables: cardiac output, heart rate, distribution of pulmonary blood flow, pulmonary blood volume, and functional residual capacity. During prolonged exposure to hypogravity, some of the above variables will shift toward normal 1-g values. The altered resting cardiovascular values will affect the subject's ability to respond to exercise. Return to 1-g will be accompanied by shifts in cardiovascular function, such that values measured immediately following reentry will differ from preflight data.

EXPERIMENT PLAN:

Preflight

Five human subjects, all members of the flight crew, will be trained to perform both the basic resting procedures and exercise procedures prior to beginning preflight measurement sessions. There will be four preflight data acquisition sessions on days 7, 5, 3, and 1 prior to launch. Each subject will perform the 30-minute measurement sequence once each day. The data gathered from these tests will be used as baseline values against which flight data will be evaluated to investigate cardiovascular and respiratory changes.

Inflight

The same five subjects will perform the 30-minute test sequence once as soon as practical after launch, once again later on Mission Day (MD) 1, and once daily thereafter for the duration of the mission. The experiment session will be completed at the approximate same time each day and preceded by an hour of non-strenuous work.

Postflight

As soon as possible upon return, each subject will perform the 30-minute test sequence. To establish the rate of return to preflight baseline, additional tests will be conducted to gather data using the same procedures again on R+1, R+7, R+14, and R+21 days.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: This investigation remains basically unchanged. The Experiment Requirements Document (ERD) adds clarifying detail. The PI now requests 31 hours of crew time. In general, the experiment is now better defined.

SCIENCE: This category 1 experiment seeks new information to provide an integrated and comprehensive assessment of cardiopulmonary function in zero-g, both at rest and during exercise. Of particular value for furthering our understanding of the effects of fluid shifts and cardiovascular deconditioning will be the measurements of cardiac output during the acute and adaptive phases of flight. Additional measurements of interest include stroke volume, lung tissue volume, functional residual capacity, tissue gas tensions, and effects of shifts in ventilation and perfusion. These objectives and test measurements of Farhi's proposal are complementary to those of West's (198) cardiopulmonary study and Blomqvist's (294) cardiovascular

study. Because this experiment utilizes new flight hardware to examine an important aspect of physiological function belived to be altered in the space environment, it directly addresses many of the announcement of opportunity, payload and program objectives.

The non-invasive rebreathing technique for measuring cardiac output, developed by the PI, uses no special tracer gas. It is, therefore, recommended that this method replace Dr. Blomqvist's similar procedure which uses the more environmentally hazardous tracer gas acetylene. Although the Farhi technique is less widely known and utilized than acetylene rebreathing, published data show excellent correspondence between the two techniques. It is also recommended that Drs. Blomqvist and Farhi share the same protocol and hardware. These changes should enhance the principal investigator's which have a high likelihood of being successfully implemented.

EQUIPMENT: This investigation does not supply any unique experiment hardware. LSLE required are the cardiopulmonary analyzer system, the physiological monitoring system and the bicyle ergometer. These items are planned LSLE.

SUMMARY: This experiment is highly complementary to that of Blomqvist (781294) and can provide the capability to perform frequent, non-invasive estimates of cardiac output on the LS-1 mission. The advantage of the Farhi technique stems from the fact that no foreign trace gas is required, such as acetylene. It is recommended that the cardiac output and related cardiopulmonary parameters of this proposal will be substituted for those proposed by Blomqvist (781294). The definition phase produced a comprehensive management and cost plan which provides confidence that this experiment can be successfully implemented. It is recommended that this proposal tentatively be selected for flight on the understanding that this proposal will be combined with Blomqvist, there is a reduction in crew time from that requested, and that Dr. Farhi's research objectives will be largely driven by the Blomqvist experiment.

FLOW CHART AND MEASUREMENT TABLE FOR EXPERIMENT #066- FARHI

TESTS	Preflight				Launch	Inflight								Launch	Postflight				
	L -7	L -5	L -3	L -1		L +2H	L +12H	L +1D	L +2	L +3	L +4	L +5	L +6		R +0	R +1	R +7	R +14	R +21
CARDIOPULMONARY STATUS: Using Rebreathing Technique and Bicycle Exercise																			
Metabolic: O_2 Uptake																			
CO_2 Output																			
Gas Exchange Ratio																			
Circulatory: Cardiac Output																			
Heart Rate(continues)																			
Stroke Volume																			
Arterial Blood Pressure	ALL TESTS WILL BE PERFORMED ON 5 SUBJECTS ON EACH DAY INDICATED ABOVE.					ALL TESTS WILL BE PERFORMED ON 5 SUBJECTS ON EACH DAY INDICATED ABOVE.									ALL TESTS WILL BE PERFORMED ON 5 SUBJECTS ON EACH DAY INDICATED ABOVE.				
Pulmonary: Minute Volume																			
Tidal Volume and Expiratory PO_2 and PCO_2																			
Functional Residual Capacity																			
Pulmonary Tissue Volume																			
Arterial-end Tidal PCO_2 Difference																			
Integrated Parameters:																			
Mixed Venous O_2 and CO_2 Pressures and Concentrations																			

PROPOSAL NUMBER: 781022

TITLE: Influence of Weightlessness Upon Autonomic Cardiovascular Controls

SPECIMEN: Human

PRINCIPAL INVESTIGATOR: Eckberg, D. L., M.D.
AFFILIATION: Medical College of Virginia, Richmond, VA

CoIs/AFFILIATIONS: Musgrave, Ph.D., MCV

BACKGROUND: Dr. Eckberg observed that nearly all astronauts, including those whose period of weightlessness was as short as nine hours, experience some degree of orthostatic hypotension upon return to earth. Disruption of normal baroreceptor reflex control could be an important factor in understanding these observations. This proposal wishes to examine baroreceptor activity in a direct, non-invasive manner.

Prior NASA grant support has led to the development and evaluation of a prototype neck cuff system for quantifying baroreceptor sensitivity. The value of the information obtained from this experiment will be enhanced if this effort is performed in conjunction with complementary studies of Dr. Blomqvist who proposed a pharmacological titration of baroreceptor sensitivity.

PI OBJECTIVES: (1) To measure, serially, and non-invasively, sinus node responses of Payload Specialists to changing levels of carotid sinus distension, before, during, and following space flight. (2) To detect shifts of baroreceptor-cardiac reflex threshold pressures, slopes, and saturation pressures during weightlessness. (3) That baroreflex impairment during space flight is dynamic, and that a circadian fluctuation of response patterns may be demonstrable. (4) To relate changes of baroreflex responses to the development of orthostatic intolerance during weightlessness.

PI HYPOTHESES: That space flight leads to an impairment of normal arterial baroreflex control mechanisms, which is manifested by major shifts of all elements of the baroreceptor stimulus-sinus node response relation. That impaired arterial baroreflex mechanisms contribute to post-flight orthostatic hypotension. That post-flight improvement of orthostatic tolerance occurs in parallel with improvement of baroreflex responses.

EXPERIMENT PLAN:

Preflight

Preflight data will be collected on four crewmen (15 min/subject) at L-30, -15, -5, -4, and -3 days. At each session, the crewmen will be fitted with a neck pressure chamber which is used with a nasal thermistor, ECG electrodes, and an indirect arterial blood pressure measurement system. A series of external pressure pulses, both above and below ambient, will be applied to the neck to stimulate the carotid baroreceptors. Heart rate and blood pressure will be monitored during and following the application of the pressure pulses. Prior to each session, blood samples will be collected from each crewman for catecholamine analysis.

Inflight

Inflight data will be collected on four crewmen on each mission day. The data collection sessions are the same as described in the preflight period except that blood samples for catecholamine analyses will be collected only on Mission Days (MD) 2, 4, and 6.

Postflight

Postflight data will be collected daily on four crewmen on R+0 through R+7. The data collection sessions are the same as described in the preflight period except blood samples will be collected only on R+0, 1, 2, and 6.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The protocol for this experiment has been simplified. The PI has devised a protocol that is automated, no longer requires lower body negative pressure, and requires less crew time than originally proposed. Measurements are now taken in the threshold region of the sigmoidal stimulus-response relation. A blood draw has been added to allow correlation of physiological baroreflex changes with changes of background sympathetic activity, as reflected by plasma catecholamine levels. The study can now accommodate women subjects with the addition of a supporting study to determine variation of baroreflex responsiveness in menstruating women. In general, the experiment has been better defined and has a more realistic management approach.

SCIENCE: This proposal utilizes an innovative and non-invasive technique to investigate baroreceptor function (carotid sinus node) as a basic cardiovascular reflex control mechanisms. In conjunction with another recommended cardiovasuclar experiment (Blomqvist 781294), it has the potential to reveal, for the first time inflight, autonomic factors responsible for the orthostatic hypotension noted in astronauts upon return to Earth. This experiment highly complements Blomqvist's proposed pharmacological assessment of autonomic function. The available evidence to date indirectly suggests a role of autonomic impairment or dysfunction (in addition to diminished blood volume) in the etiology of orthostatic intolerance. The proposal, therefore, constitutes a logical extension of previous flight research in its search for mechanisms responsible for degrading human performance in weightlessness. Thus, this category 1 proposal was given high scores by all review panels and satisfies the primary announcement of opportunity, payload, and program objectives.

Prior NASA grant support has led to the development and evaluation of the system proposed for quantifying baroreceptor sensitivity. The definition phase was successfully completed and did not identify any problems related to the development of this experiment for the LS-1 payload. There are no scientific concerns regarding this experiment and there is a high likelihood of success in meeting the proposed objectives. The only modification to which the PI must agree will be the omission of the circadian analysis of baroreflex behavior (morning and afternoon sessions), a change required in order to reduce crew-time allotment to a more reasonable level.

EQUIPMENT: This experiment requires the LSLE physiological monitoring system, the rack mounted centrifuge, the inflight blood collection system, minioscilloscope, and freezer. The PI will provide his unique neck chamber device, pressure control system, and electronic control system. The LSLE items are in LSLE inventory and are under development or in the procurement cycle.

SUMMARY: This proposal addresses alterations of normal baroreceptor reflex control and will be an important factor in understanding the orthostatic intolerance noted in astronauts upon return to Earth. Prior NASA support has led to the development and evaluation of the system proposed for quantifying baroreceptor sensitivity. The definition phase was successfully completed and did not identify any problems related to the development of this experiment for the LS-1 payload. This experiment complements the studies of Blomqvist (781294) who proposed a pharmacological assessment of baroreceptor sensitivity. It is recommended that this proposal tentatively be selected for flight on the understanding that the multiple measurements seeking to demonstrate a circadian fluctuation of the baroreceptor-cardiac response may have to be curtailed due to inadequate crew time.

FLOW CHART AND MEASUREMENT TABLE FOR EXPERIMENT #022 - ECKBERG

TESTS	Preflight					Launch	Inflight						Recovery	Postflight						
	L -30	L -15	L -5	L -4	L -3	L +0	L +1	L +2	L +3	L +4	L +5	L +6	R +0	R +1	R +2	R +3	R +4	R +5	R +6	R +7
BARORECEPTOR - CARDIAC REFLEX TEST:																				
NECK CHAMBER PRESSURE																				
ELECTROCARDIOGRAM (Lead with Prominent P Waves)	ALL TESTS PERFORMED ON 4 CREWMEN ON ALL DAYS INDICATED ABOVE						ALL TESTS PERFORMED ON 4 CREWMEN ON ALL DAYS INDICATED ABOVE (EXCEPT AS SHOWN)							ALL TESTS PERFORMED ON 4 CREWMEN ON ALL DAYS INDICATED ABOVE (EXCEPT AS SHOWN)						
RESPIRATION (Nasal thermistor)																				
BLOOD PRESSURE (cuff)																				
PLASMA SAMPLE (Catecholamine Levels)							4		4		4		4	4	4				4	

PROPOSAL NUMBER: 781198

TITLE: Pulmonary Function During Weightlessness

SPECIMEN: Human

PRINCIPAL INVESTIGATOR: West, J. B., M.D., Ph.D.
AFFILIATION: University of California at San Diego, CA

CoIs/AFFILIATIONS:
D. B. Michels, Ph.D./University of California at San Diego, CA
P. D. Wagner, M.D./University of California at San Diego, CA
C. F. Sawin, Ph.D./Perkin-Elmer Corporation, Pomona, CA

BACKGROUND: Dr. West plans to determine the importance of a gravitational environment on pulmonary function in humans. The investigation will explore the distribution of pulmonary blood flow and regional pulmonary ventilation. Further, Dr. West intends to expand upon the results obtained performing similar studies onboard aircraft during transient 0-g exposure in Keplerian flight trajectories.

The general approach is to have each subject performing a set of six non-invasive measurements of pulmonary function at specific intervals during the Spacelab mission. These inflight measurements will culminate 10-years of NASA support in this research project.

The scientific yield from these studies will clearly establish gravity-related changes in pulmonary function. Hypotheses are well established and can be proven or disproven on this mission.

Minimal pulmonary function data (vital capacity and $^{V}E_{max}$) were obtained during Skylab due to limitations in available instrumentation. The proposed studies relate to Announcement of Opportunity (AO) criteria "to investigate significant biological phenomena which may occur during and/or after exposure to the space environment," and "to ensure human health, safety, well-being and effective performance in space flight."

PI OBJECTIVES: The objective of the study is to compare pulmonary function during weightlessness with pulmonary function during normal gravity. The following aspects of pulmonary function will be examined: distributions of ventilation and perfusion; diffusing capacity; lung mechanics; gas exchange; and pulmonary bloodflow.

PI HYPOTHESES: Many aspects of pulmonary function will be greatly altered during weightlessness, including the distribution of ventilation and perfusion, diffusing capacity, pulmonary capillary volume, airway closure and lung mechanics. Gravity is the major cause, but not the only cause of non-uniformity in the distributions of ventilation and perfusion at 1-g. Pulmonary function may temporarily be degraded upon return to a positive gravitational loading following exposure to weightlessness.

EXPERIMENT PLAN:

Preflight

A series of six standard pulmonary function tests (PFT's) will be performed by each of three human subjects at intervals of 5, 4, 3, 2, and 1 months and 1 week prior to launch. Each subject will perform the 45 minute sequence on himself once in an upright, seated position and once in a supine position during each session. The three subjects will be the same crewmen who will perform the pulmonary function testing inflight and postflight.

Inflight

The three subjects who measured their pulmonary function preflight will perform the same series of six tests inflight on Mission Days (MD) 2, 4, and 6. The pulmonary function test sequence will be performed only once by each crewman on each of these days.

Postflight

As soon as possible upon return and landing (within 12 hours) each of the three subjects who performed the PFT inflight will measure their pulmonary function by completing the same series of six standard tests. Each crewman will perform the sequence once in a seated, upright position and once in a supine position. These procedures will again be repeated on days 2, 5, and 14 following return.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The original proposal, which was a comprehensive and thoughtful effort, remains basically unchanged. Some clarifying detail was added.

The management and cost proposal remains basically unchanged in terms of total cost proposal.

SCIENCE: The specific contribution of these studies to LS-1 is a careful analysis of acute changes in cardiopulmonary function during human exposure to weightlessness. Although there are no known problems in pulmonary function, it is expected that changes in lung mechanics and ventilation and perfusion distribution will occur. These have never been appropriately evaluated. Dr. West's comprehensive pulmonary function study offers major new information in the context of basic human gravitational biology and is, therefore, in accord with the primary announcement of opportunity, payload and program objectives.

Experimental hardware has been developed over a period of years under NASA support and prototype hardware has been evaluated during ground-based Spacelab mission simulations. The procedures have been tested on aircraft in parabolic flight and they are well defined and extremely efficient. There are no scientific concerns with this experiment. This experiment can share common hardware (cardiopulmonary analyzer system) with the studies of Farhi (066) and Blomqvist (294), two other highly recommended experiments. The data concerning pulmonary blood flow, tissue volume, and other pulmonary parameters will also be complementary to these other experiments.

EQUIPMENT: No P.I. provided equipment is required for this investigation. LSLE required the cardiopulmonary analyzer system, multichannel strip chart recorder, microcomputer, and the gas analyzer - mass spectrometer.

SUMMARY: This proposal is highly relevant to the AO and payload objectives. These studies will identify those gravity-related changes in pulmonary function related to weightless exposure. The definition phase confirmed the high state of readiness of this proposal. This experiment will share LSLE hardware with other proposers (Blomqvist, 781294 and Farhi, 781066). This is a major experiment in human gravitational biology and has high scientific priority. It is recommended that this proposal tentatively be selected for flight on the understanding that the PI agrees to combine scientific objectives with Blomqvist, to accept a budget reduced from that requested, and to share hardware with Farhi and Blomqvist.

FLOW CHART AND MEASUREMENT TABLE FOR EXPERIMENT #198 - WEST

TESTS	Preflight						Launch	Inflight			Recovery	Postflight			
	L -150	L -120	L -90	L -60	L -30	L -7		L +2	L +4	L +6		R +0	R +2	R +5	R +14
PULMONARY FUNCTION TESTS															
RESTING GAS EXCHANGE AND VENTILATION - PERFUSION INEQUALITY: Resting O_2 consumption, CO_2 output, respiratory exchange ratio, ventilation end-tidal PCO_2 and PO_2 ventilation perfusion ratio	ALL TESTS PERFORMED ON 3 CREWMEN ON DAYS INDICATED ABOVE							ALL TESTS PERFORMED ON 3 CREWMEN ON DAYS INDICATED ABOVE				ALL TESTS PERFORMED ON 3 CREWMEN ON DAYS INDICATED ABOVE			
PERFUSION INEQUALITY: Regional inequality of pulmonary blood flow															
DIFFUSING CAPACITY FOR CARBON MONOXIDE (^{18}O labeled CO)															
PULMONARY BLOOD FLOW (N_2O rebreathing) AND RESIDUAL VOLUME (He dilution)															
DISTRIBUTION OF VENTILATION (single breath N_2 washout)															
FORCED EXPIRATION SPIROMETRY: Forced vital capacity, forced expiratory volume, maximum mid-expiratory flow rate, flow-volume curve															

PROPOSAL NUMBER: 781248

TITLE: Cardiovascular Adaptation of White Rats to Decreased Gravity of Space Shuttle/Spacelab Inflight Conditions

SPECIMEN: Rat

PRINCIPAL INVESTIGATOR: Popovic, V. P., D.Sc.
AFFILIATION: Emory University, Atlanta, GA

CoIs/AFFILIATION:
P. Popovic, Ph.D./Emory University, Atlanta, GA

BACKGROUND: Cardiovascular alterations during exposure of man to weightlessness and the subsequent readaptation after return to Earth are known to occur, but the underlying mechanisms are not understood. Some of the changes observed include shifts in body fluid, salt and water loss, increases in heart rate, and a reduction in plasma volume and red blood cell mass. These physiological adjustments result in orthostatic intolerance and a decreased exercise tolerance. Insight into the mechanisms governing cardiovascular alterations may be obtained from bedrest studies in man or hypokinesia in animals. The purpose of this study is to document the changes to weightlessness that take place in rats and compare them to the rat hypokinesia model.

PI OBJECTIVES: To investigate and quantify the circulatory changes that occur in the rat during weightlessness. To verify that the circulatory effects shown in the rat head-down hypokinesia model are an accurate representation of effects shown in weightlessness. To chart the time course of the circulatory changes that occur during readaptation to unit gravity.

PI HYPOTHESES: The adaptive circulatory changes of rats exposed to weightlessness are a model for the changes observed in man. The adaptive circulatory changes observed in hypokinetic head-down rats are a model for the changes seen in weightless men and possibly in weightless animals. The circulatory changes following release from hypokinesia are a model for the changes seen in the crewmen on return to unit gravity.

EXPERIMENT PLAN:

Preflight

Male Sprague-Dawley rats will be reared at KSC on the flight diet. When the animals reach approximately 180 grams body weight, 14 animals will be implanted with aortic and right ventricular cannulas. The animals will then be separated into two functional groups of six each and two back-up animals:

Group A: Cardiovascular testing at rest (pre-, in-, and postflight)
Group B: Cardiovascular testing during exercise (pre-, postflight)
Group C: Surgery backup. No further use.

After recovery from surgery and acclimatization to the Research Animal Holding Facility (RAHF), baseline measurements are made.

The parameters to be measured on Group A (three sessions with one day between sessions) are aortic pressure, right ventricular pressure, cardiac output, blood volume, hematocrit, body mass. The same parameters will be measured for Group B (three sessions with one day between sessions) during steady-state exercise on a treadmill operating at 10 meters/minute. Group B animals will be tested on alternate days and at rest following an injection of norepinephrine (measure pressures) and epinephrine (measure oxygen consumption).

Inflight

Group A animals will be tested on days 2, 4 and 6 of flight. The same parameters are measured as were measured preflight. Group B animals are not tested in-flight.

Postflight

Group A animals will be tested on days 1, 2, 4, 7, 10, 13 during recovery, using the same regimen as for preflight and flight, until the animals have returned to their preflight levels with respect to the measured cardiovascular parameters. Group B animals will be tested on days 1, 3, 5, 8, 11, 14 during recovery, using the same regimen as for preflight, until they have returned to their preflight levels.

Ground Control

A nominal flight profile control group of animals, identical in all respects to the flight subjects, will undergo the same preflight and postflight testing as the flight subjects. Instead of flight, these animals will be housed in a ground-based RAHF that simulates the flight RAHF. The entire test will occur postflight, after testing has been completed on the flight specimens.

A hypokinesia control group of animals, identical in all respects to the flight subjects, will undergo the same preflight and postflight testing as the flight subjects. Instead of flight, these animals will be made hypokinetic (using head-down restraint) for the same length of time as flight. The entire test will occur postflight but will not be concurrent with the normal flight profile control.

Measurements

Type	Units
Aortic pressure**	mmHg
Right ventricular pressure**	mmHg
Cardiac output**	ml/min
Blood volume**	ml
Hematocrit**	%
Total body mass**	g
Oxygen consumption	ml/min
Total white blood cell count	count
Differential red blood cell analysis	count
Right atrial pressure**	mmHg

Type	Units
Cardiac index**	ml/min/g
Heart rate**	beats/min
Stroke volume**	ml
Temperature**	°C
Humidity**	%
Noise**	dB
Vibration**	g
Light cycle**	h
Atmospheric pressure*	mmHg

* Data taken during flight procedures only.
** Data taken during flight and ground procedures.
All other data taken on the ground.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: As proposed, the experiment would use 12 rats for flight, 12 as unrestrained vivarium controls, and 12 as hypokinetic controls. Each group would be exposed to treadmill exercise preflight and postflight with concurrent measurement of cardiovascular parameters. In-flight measurements would occur on days 1, 5 and 10 of a 10-day flight. No in-flight exercise is planned.

The vivarium control was replaced with a nominal flight profile control in the Experiment Requirements Document (ERD). In addition, the 12 rats in each category were further divided into 2 groups of 6 each (Group A, Group B). Group A rats are normal, non-exercised animals, while Group B rats are subjected to treadmill exercise preflight and postflight. The Group B flight animals are not tested during flight. The measurements list was extended to include hematocrit, blood volume and body mass. In-flight measurements are to be taken on days 2, 4 and 6 of a 7-day flight.

SCIENCE: The experiment proposes to quantify the central cardiovascular changes that occur in the rat as a result of weightlessness and to validate the head-down hypokinesia model. The proposal directly addresses a known problem of spaceflight from an animal model standpoint and is consistent with payload and program objectives. The experimental design is such that those objectives will be met. Appropriate controls have been planned. With Popovic's extensive experience with the experimental preparation and his virtual independence of flight hardware, his chances of gaining results are very high. A minor concern is that the technique for cardiac output determination and the requirement to measure blood volume both involve taking blood samples, which will reduce the blood volume and change the cardiac output unless great care is taken to replace the removed volume.

It is proposed that the experiment be shared with that of Hutchins (781166). If both investigators approve that plan, the animals would be implanted with electromagnetic flow probes for the cardiac output determination, eliminating the concern about taking blood samples. The two experiments would be merged and six animals would be used for flight. These animals would be implanted with dorsal microcirculatory chambers, EM flow probes and arterial catheters.

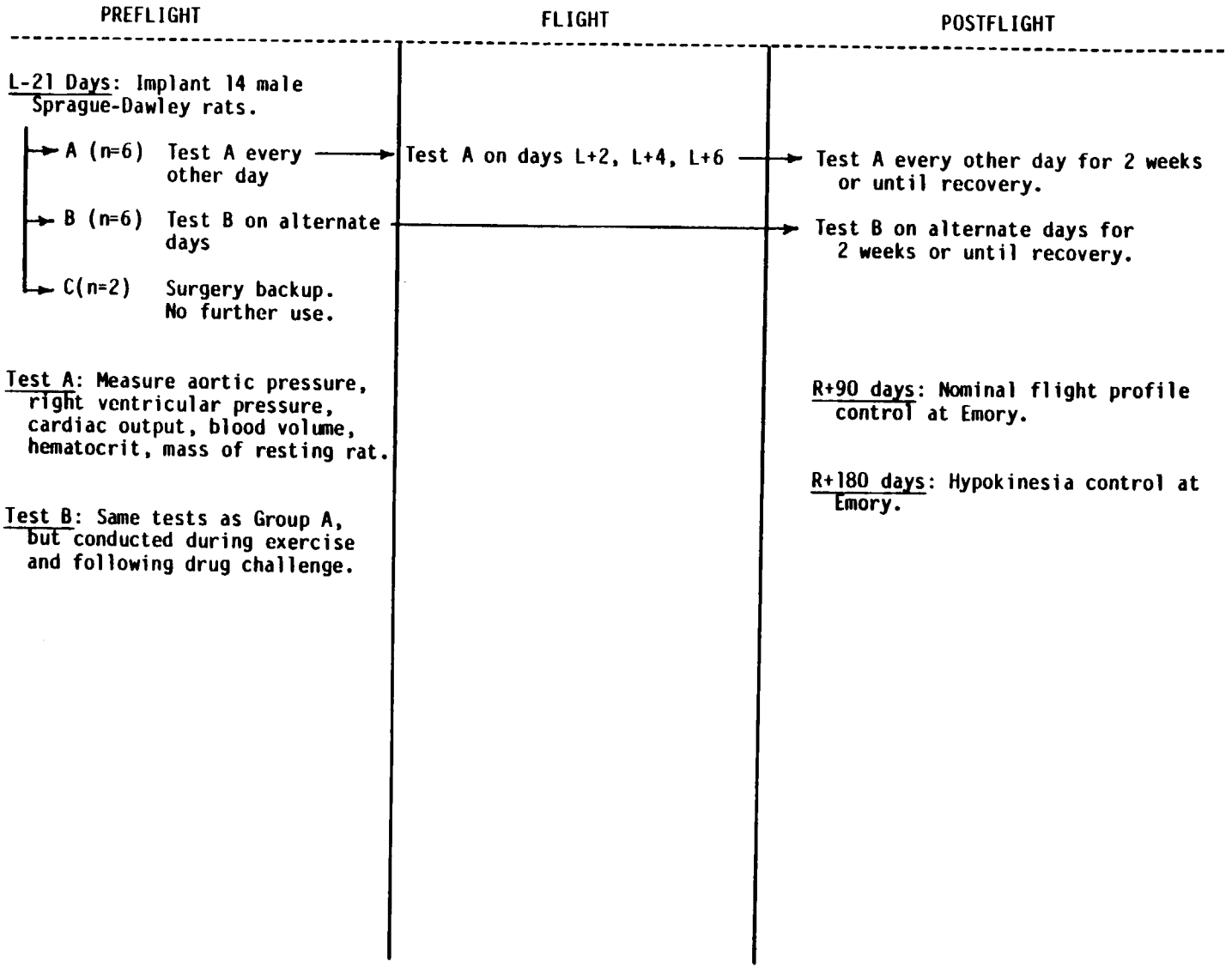
Popovic's six exercise-conditioned rats would not be utilized. These changes strengthen both experiments and eliminate duplication.

EQUIPMENT: The PI's hardware requirements are simple and straightforward. It is likely that the pressure transducers, pressure calibration reference, and signal conditioners will all be part of the LSLE animal physiological signal conditioner assembly. This equipment will be the same hardware that is listed under Hutchins' experiment; for shared experiments, duplications have been eliminated.

The experiment unique equipment will be a rat holding box, cannula sealer, equipment kit, and freezer container. The LSLE will be 1/4 RAHF, GPWS, -20°C freezer, 4°C refrigerator, SMMI, mini-oscilloscope, hematocrit centrifuge, voice recorder, bioradiation storage container, DEMS, animal physiologial signal conditioning system including 2 pressure transducers and a pressure calibration reference, and an event timer.

SUMMARY: This experiment represents an excellent study of cardiodynamics. It seeks to quantify the central cardiovascular changes that occur in the rat as a result of weightlessness. The proposal directly addresses a known problem and seeks to validate the rat as a useful human surrogate. Its likelihood of success is excellent. It is recommended that this experiment tentatively be selected for flight on the understanding that this proposal be combined with that of Hutchins (781166), to use an aortic flow probe to measure cardiac output, and to agree to utilize fewer animals including the exclusion of the exercise-conditioned rats.

EXPERIMENT FLOW DIAGRAM - ES 248



PROPOSAL NUMBER: 781166

TITLE: Correlation of Macro- and Microcirculatory Alterations During Weightlessness

SPECIMEN: Rat

PRINCIPAL INVESTIGATOR: Hutchins, P. M., Ph.D.
AFFILIATION: Bowman Gray School of Medicine, Winston-Salem, NC

CoIs/AFFILIATIONS:
J. W. Dusseau, Ph.D./Bowman Gray School of Medicine, Winston-Salem, NC

BACKGROUND: During spaceflight cardiac output is presumed to increase and increased tissue perfusion may also occur. In hypertension an increased cardiac output with overperfusion of body tissues beyond metabolic demands leads to a long term reduction in the number of arterioles and an increase in the number of venules. Thus mechanisms leading to cardiovascular deconditioning during exposure to weightlessness may share common factors with the mechanisms leading to the development of hypertension. If the mechanisms are similar, and an increased number of venules develop during spaceflight, these new venules may initially have poorly developed vascular tone. Consequently, during return to Earth, the hydrostatic pressure changes may not be countered by compensatory venoconstriction which could account for the observed orthostatic intolerance. A change in ratio of arterioles to venules would also favor fluid reabsorption into the vascular space and contribute to an already increased venous return. An altered distribution of vessels could also influence blood pressure.

PI OBJECTIVES: To measure central cardiovascular changes during weightlessness. To record microcirculatory alterations during weightlessness. To relate the observations to filtration/reabsorption at the tissue level and fluid/electrolyte balance of the whole animal. To observe the effects of weightlessness on the rheology of the formed elements of blood, red blood cell deformability and platelet and white cell appearance and flow patterns. To determine the overall cardiovascular compensatory mechanisms during space flight.

PI HYPOTHESES: There will be cephalic shift of blood volume during space flight. There will be an increased cardiac output during space flight, resulting in an overperfusion of tissue. There will be a decreased tone of venules, an increased tone of arterioles and an increased vasomotion in skeletal muscle during space flight. There will be long-term autoregulation in skeletal muscle due to overperfusion, as evidenced by an increased number of venules and a decreased number of arterioles.

EXPERIMENT PLAN:

Preflight

Thirty male, Wistar, 4-week old rats will be shipped to KSC to arrive 4 weeks prior to launch. They will be housed in Research Animal Holding Facility (RAHF)-like cages and be fed the flight diet. The rats will be implanted (see below) in the weeks prior to flight. An overall 80% success rate is

assumed for the combined implants, which will leave 24 rats suitable for experimentation. These will be split into 4 groups of 6 each: (1) a flight group; (2) a vivarium control; (3) a nominal flight profile control with noise and vibration, and (4) a flight backup group. The flight backup group, if not used for flight, plus extra surgery survivors, will be used as additional vivarium controls.

Surgery schedule: In the third week prior to launch, all rats will be implanted with the dorsal microcirculatory chamber. One week later, survivors will be implanted with an electromagnetic flow probe around the aorta. The connector will be externalized at the back of the neck. In the last week before launch, 20 of the survivors will be implanted with a catheter in the femoral artery, externalized to the back of the neck. Eighteen are expected to have fully functional implants. On launch day, the remaining rats will be implanted with femoral catheters. This last group will comprise the flight backup group and will accommodate a launch hold of up to a week.

Test schedule: Rats will be weighed daily. Following each surgical intervention, the rats will be given three days to recover before measurements are taken. After the dorsal microcirculatory chamber is implanted, photomicroscopy will be done daily. After the flow probe is implanted, daily flow measurements will be made in addition to the photomicroscopy. After the rats are catheterized, pressure measurements will be included in the daily testing. In addition, hematocrit and blood volume will be determined just prior to flight. An orthostatic tolerance test will be performed, with pressure and flow measurements being made with the rat at rest, standing, and again at rest.

Inflight

The ground control animals (vivarium and nominal flight profile) will undergo the same testing as the flight animals.

On days 2, 4, and 6 of a 7-day flight, each animal will have its arterial pressure and aortic flow measured; a 35mm photomosaic will be made of the dorsal microcirculatory chamber; videomicroscopy will be achieved at each of 5 pre-determined landmarks in the microcirculatory chamber; and the animal's mass will be determined. These measurements will be taken at the same time each session, corresponding to the times used in establishing the preflight baseline data for each animal.

Postflight

The flight animals will be processed first, followed by the nominal flight profile and vivarium controls. Approximately 3 animals can be processed per day.

At the next diurnal cycle time after recovery, the inflight tests will be repeated on all animals. Blood volume and hematocrit will be determined. Orthostatic tolerance testing will be done. Three of the animals will be used for *in vivo* microscopy of the cremaster muscle, followed by latex injection of the cremaster blood vessels and dissection for later analysis. On the following day, the remaining 3 flight animals will be processed. Control animals will be processed on successive days.

Ground Controls

Two synchronous control studies will be performed. The vivarium control will allow direct comparison of the data from the flight rats with data from 1-g animals. The nominal flight profile control with noise and vibration will allow the stress effects of the Spacelab habitat to be taken into account. In addition, a group of animals in the PI's lab will be made hypokinetic (head-down restraint) for a direct comparison of the flight data with data derived from this well-known fluid shift model.

Measurements

Type	Units
Cardiac output**	ml/min
Arterial pressure**	mmHg
Total body mass**	g
Hematocrit	%
Blood volume	ml
Vasculature photomosaic**	frame
Vasculature photo	frame
Vasomotion videotape**	micron/beat
Orthostatic tolerance	ratio
Heart rate	beat/min
Stroke volume	ml
Peak flow velocity	ml/min_2
Peak flow acceleration	ml/min^2
Number arterioles, venules	count
Diameter arterioles, venules	micron
Tone of arterioles, venules	N/A
Rheology of RBCs, WBCs, platelets	N/A
Cremaster vasculature photomosaic	frame
Cremaster vasomotion videotape	micron/beat
Number cremaster arterioles, venules	count
Diameter cremaster arterioles, venules	micron
Tone cremaster arterioles, venules	N/A
Rheology cremaster RBCs, WBCs, platelets	N/A
Temperature**	°C
Humidity**	%
Acceleration*	g
Vibration**	g
Noise**	dB
Food consumption**	g
Water consumption**	ml
Light cycle**	h
Atmospheric pressure**	mmHg

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* Data taken during flight procedures only.
** Data taken during flight and ground procedures. All other data taken on the ground.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The study proposed to use five rats, with pressure and flow measurements made daily during flight, and videomicroscopy and photography of the dorsal flap made every other day. Five rats were proposed for a vivarium control, and five for a nominal flight profile control.

The sample size of each group was increased to six animals in the ERD. Preflight supporting studies were defined and included a study of the effects of acceleration on the dorsal flap and studies of the cardiovascular changes occurring as a result of mass measurement and head-down hypodynamia. Inflight measurements were defined to occur on mission days 2, 4 and 6. Hematocrit and blood volume were added to the measurements list preflight and postflight, and body mass measurements inflight.

SCIENCE: The investigator proposes to determine the extent of change of peripheral versus central blood volume during exposure to weightlessness in the rat. The experiment is well-designed. The approach to the investigation is based on considerable ground-based information and the results of earlier spaceflights. If weightlessness affects the microcirculatory bed, this investigation will be able to detect those changes. This is essentially two experiments in one, as it will document the central cardiovascular effects as well as the peripheral microcirculatory effects of weightlessness in the rat. The surgical interventions required to implant the flow measurement and other devices will be carefully monitored and controlled in the flight animals. The devices have been implanted in animals in the investigator's laboratory for several years.

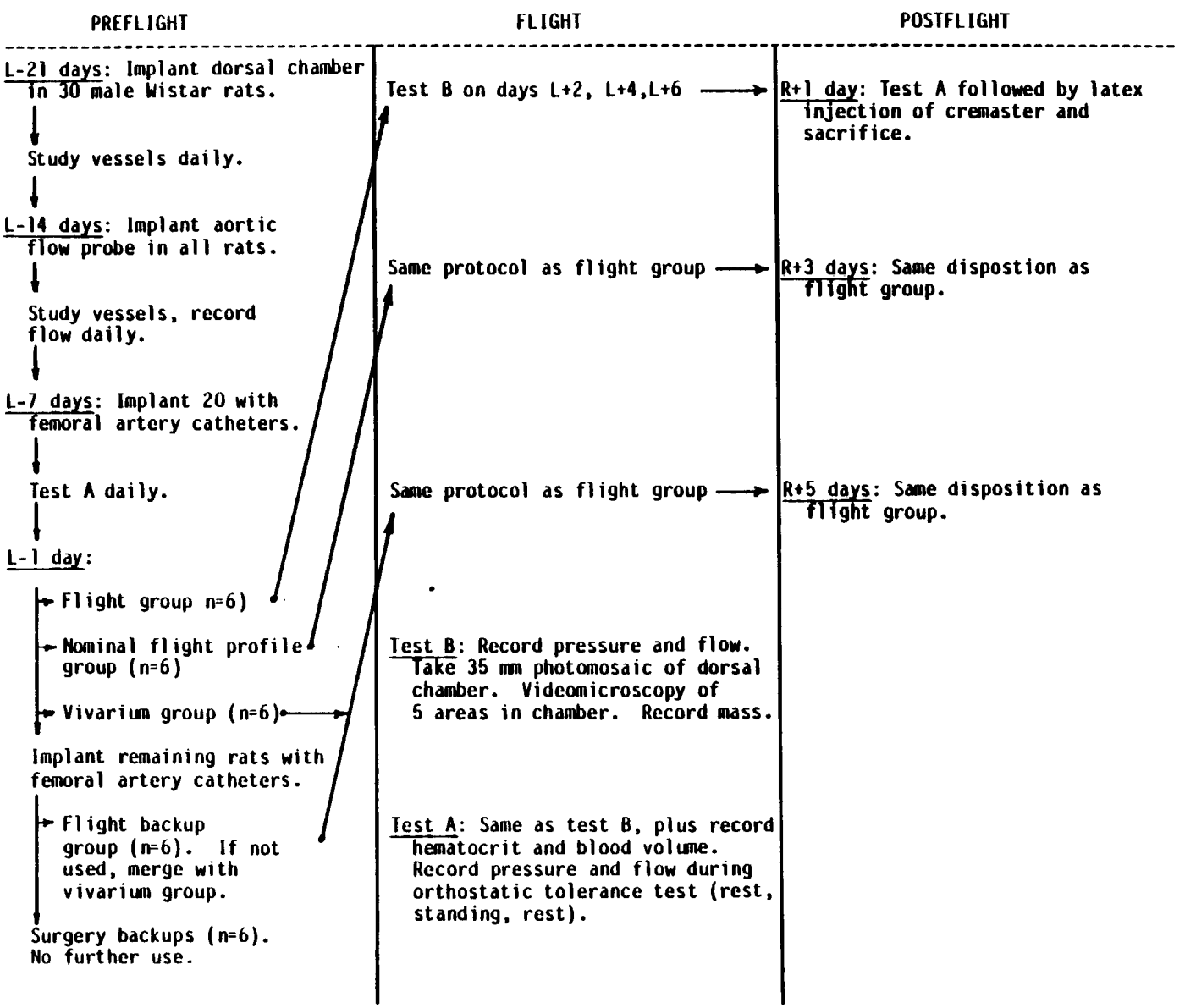
Definition phase activity emphasized the determination of problems associated with the implant and monitoring techniques. The scientists and engineers assigned to this experiment have concluded that the technique is viable and the data will likely be exciting and significant. In order to accomplish the selection of this experiment for the dedicated spacelab, the investigator would have to agree to combine efforts with another rodent cardiovascular experiment and share the flight animals. As a result of the definition phase activities, this experiment is in a higher state of readiness than originally anticipated.

EQUIPMENT: PI has had experience with this experiment in his lab and has used similar equipment and the same main vendor before. The investigator has asked that NASA provide a video camera and 35 mm camera that will interface with the compound microscope. Experiment unique equipment for the experiment is as follows: a video camera; signal conditioner; electromagnetic flowmeter; dissection kit and other surgical equipment. The LSLE required for this experiment is: RAHF; 35-mm camera; GPWS; microcomputer; compound microscope; mini-oscilloscope; video monitor; SMMI; video recorder; voice recorder; videotape cassettes; DEMS; and animal physiological signal conditioning system.

SUMMARY: This is a sound, innovative proposal to determine the shifts in blood volume theorized to occur between the central and peripheral components of the circulation. It is the only experiment proposed which directly examines the effects of weightlessness on the peripheral circulation. This information complements the extensive measurements of central cardiovascular function by other investigators. A synergistic effort between this research group and that of Popovic (781248) will enhance both experiments. Both seek

to validate the rat as an appropriate animal model of cardiovascular adaptation to weightlessness. The equipment required is predominently LSLE. It is recommended that this proposal tentatively be selected for flight on the understanding that Hutchins' instrumented animals will be shared with Popovic and that there will be no sacrifice immediately postflight.

EXPERIMENT FLOW DIAGRAM - ES 166



PROPOSAL NUMBER: 781072

TITLE: Vestibular Experiments in Spacelab

SPECIMEN: Human

PRINCIPAL INVESTIGATOR: Young, L. R., Ph.D.
AFFILIATION: Massachusetts Institute of Technology, Boston, MA

CoIs/AFFILIATIONS:
R. E. Malcolm, Ph.D./Defense and Civil Institute, Ottawa, Canada
G. M. Jones, M.D./McGill University, Montreal, Canada
K. E. Money, Ph.D./Defense and Civil Institute, Ottawa, Canada
C. M. Oman, Ph.D./Massachusetts Institute of Technology, Boston, MA
D. G. D. Watt, Ph.D./McGill University, Montreal, Canada

BACKGROUND: Dr. Young is a well known vestibular investigator with a long association with NASA. The proposed experiment seeks to demonstrate changes in human vestibular functions associated with weightless exposure, placing special emphasis on otolith function. The experiment also seeks to provide some insight into space motion sickness by comparing head accelerations with repeated assessments of motion sickness symptomatology. A provocative test of motion sickness susceptibility will be performed inflight and a larger group of such tests will be done preflight to later correlate with the observed incidence of space sickness inflight.

The scientific yield of this experiment involves new information on otolith function in weightlessness which may provide practical insight into the management of space motion sickness.

In Skylab it appeared that the perception threshold to angular acceleration was essentially unchanged from preflight measurements. Yet, despite considerable space motion sickness early inflight, motion sickness susceptibility measured with paced head movements on a rotating chair was quite low later in the mission. Dr. Young's proposal addresses these interesting findings and represents a reasonable and appropriate extension of the research efforts on Skylab. By addressing vestibular function and space motion sickness in particular, the proposal seeks to examine two specific objectives mentioned in the Announcement of Opportunity (AO). This proposal is therefore considered of high relevance. Because of its high relevance and breadth of vestibular measurements, it is considered to be a primary vestibular experiment for the LS-1 payload.

PI OBJECTIVES: This is a highly complex human experiment which addresses a number of objectives related to vestibular adaptation to weightlessness and the etiology of space sickness. Included are pre-, in-, and postflight measurements of (1) perception of linear motion, (2) vertical, horizontal and counterrolling eye movements, (3) visually induced roll, (4) awareness of body position, (5) otolith-spinal reflexes and (6) susceptibility to motion sickness. Also included are several pre- and postflight tests of vestibular function. Several of the experiments require use of a linear acceleration device like the ESA Space Sled. This proposal is almost a repeat of SL-1 experiment 1NS102.

PI HYPOTHESIS: The sensitivity of the otolith organ response is shifted during exposure to weightlessness and that this shift carries over to the postflight experience.

EXPERIMENT PLAN:

Preflight

The "experiment" is composed of seven functional objectives (FO). Functional objective number 7 is the preflight and postflight testing of all of the experimental protocols conducted during flight. In addition, motion sickness susceptibility to a variety of provocative tests will be evaluated to determine if they are of value in predicting space sickness susceptibility on-orbit. The preflight tests (stepping, hopping, rail tests, "drop" tests) will be conducted on two crewmen (MS2, PS2). This testing will be conducted over a 10-day period 12 months before flight and on day 7 through 1 prior to flight. There will be one session/FO/man.

Inflight

The FO's to be performed during flight are the following: (1), perception of linear acceleration (sled); (2a), horizontal and vertical eye deviation during acceleration (sled); (2b), rotational eye movements (counterrolling) during acceleration (sled); (6a), provocative motion sickness testing (sled). These FO's requiring the sled will be performed continuously at 90 min/performance. Further FO's are (3), visually induced roll (rotating dome) at 45 min/performance; (4), spatial awareness/body/targets) at 50 min/performance; (5), otolith - spinal reflexes (hopping) at 24 min/peformance; and (6b), continuous head movement monitoring and recording of pertinent crew comments. Each of these FO's will be performed on two crewmen.

Postflight

As with preflight testing, postflight testing is FO number 7 consists of all the experimental protocols conducted during flight as well as additional stepping, hopping, rail tests, "drop" tests. These tests will be conducted at R+0 for 2 hrs/man, R+1 through R+5 at 0.5 hr/man, R+25 for 2 days, and R+90 for 2 days. These tests will be performed on two crewmen.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: Not applicable.

SCIENCE: Dr. Young's experiment consists of a broad battery of tests designed to provide new information concerning changes in human otolith function during weightlessness and to assess the relationship between head movement, otolith function, and space sickness during space flight. Thus, this category 1 experiment addresses a significant biological phenomenon which is closely associated with a major physiological event (space sickness) observed in humans during space flight. This experiment is fully in accord with both the program and the payload objectives, and has a high probability of being carried to successful completion.

Dr. Young originally proposed to perform parts of this experiment using a vestibular sled in Spacelab, but since such a sled is not presently planned for LS-1, those parts of this experiment dependent on the sled are not recommended for selection. The loss of these components, specifically dealing with linear acceleration perception, eye deviation and ocular counterrolling during linear acceleration, and motion sickness susceptibility during linear oscillation, reduces the scientific yield of the experiment. The remainder of the experiment still represents a broad study of high operational relevance in an area of priority.

No formal definition phase was necessary for this experiment, because essentially the same experiment has been selected and readied for flight on an earlier Spacelab mission (SL-1). Thus, the scientific protocol and flight hardware have been verified already through recent flight crew training and testing. Reflight of this important experiment provide the opportunity for improved statistical significance to be obtained through the use of a larger sample size.

EQUIPMENT: This experiment requires several items of LSLE including a microcomputer, voice recorder, EMG amplifiers, and an oscilloscope. Included as P.I. supplied equipment are a rotating dome assembly, hop station, and many associated stowed items. This equipment is being developed for SL-1 and will be reflown on LS-1.

Note: The Hop Station and associated equipment are supplied by Canadian government for use by Canadian Co-I. It would not be available if the Canadian is not chosen as Co-I.

SUMMARY: This experiment addresses both vestibular function and space motion sickness and thus is related to the first two AO objectives. The expected scientific yield of this experiment is new information on otolith function in weightlessness which may provide practical insight into the management of space motion sickness. Part of this proposed experiment involves the use of a linear accelerator which will not be available. Dr. Young can, however, explore those functional objectives that do not involve an accelerator. Only one scientific objective is eliminated, still leaving six other objectives. The anticipated scientific yield of the experiment is of high operational relevance in an area of high priority. Aspects of the experiment will be flown on SL-1. Most of the measurements involved in this experiment have considerable variance such that improved statistical significance will be obtained through increased sample size. It is recommended that this proposal tentatively be selected for flight with the exclusion of the use of the linear accelerator and the provision that the motion sickness portion that conflicts with Cowings (781195) may not be done.

FLOW CHART AND MEASUREMENT TABLE FOR EXPERIMENT #072 - YOUNG

TESTS	Preflight L (-365)	Preflight L (-7 to -1)	Launch	Inflight* Early	Inflight* Mid	Inflight* Late	Recovery	Recovery R (+0 to +5)	Recovery R (+90 to +92)
PERCEPTION OF LINEAR ACCELERATION (SLED): Motion Perception and Velocity Estimates	2	2		2	2	2		2	2
EYE DEVIATION AND OCULAR COUNTERROLLING DURING LINEAR ACCELERATION (SLED)	2	2		2	2	2		2	2
ROTATING VISUAL FIELD: Perception of Self Motion and Ocular Counterrolling	2	2		2		2		2	2
AWARENESS OF BODY POSITION AND SPATIAL LOCALIZATION	2	2		2		2		2	2
OTOLITH SPINAL REFLEXES: Calf Muscle EMG, vertical acceleration, floor contact	2	2		2	2	2		2	2
SUSCEPTIBILITY TO MOTION SICKNESS DURING LINEAR OSCILLATION (SLED)	2	2		2	2	2		2	2
PASSIVE MONITORING OF CREW HEAD MOVEMENT	2	2		2		2		2	2
PRE AND POSTFLIGHT MOTION SICKNESS SUSCEPTIBILITY TESTS: Linear Acceleration, heavy water ingestion, horizontal axis rotation, visually induced roll, left/ right reversing prisms, zero-g parabolas, headward fluid shifts	2	2						2	2
PROVOCATIVE TESTING OF ANGULAR HEAD MOVEMENTS	2	2		2				2	2
PRE AND POSTFLIGHT TESTING OF POSTURAL STABILITY	2	2						2	2

*Specific Day Not Specified

PROPOSAL NUMBER: 781195

TITLE: A Preventive Method for the Zero Gravity Sickness Syndrome: Autogenic-Feedback Training for Vestibular Symptomatology

SPECIMEN: Human

PRINCIPAL INVESTIGATOR: Cowings, P. S., Ph.D.
AFFILIATION: NASA-ARC, Moffett, Field, CA

CoIs/AFFILIATIONS:
W. B. Toscano, M.S./University of California, San Francisco, CA
N. E. Miller, Ph.D., D.Sc./Rockefeller University of New York, NY
E. R. Hilgard, Ph.D., D.Sc./Stanford University, Stanford, CA
J. Kamiya, Ph.D./University of California School of Med, San Francisco, CA
J. Sharp, Ph.D./NASA-ARC, Moffett Field, CA
T. Tanner, Ph.D./NASA-ARC, Moffett Field, CA

BACKGROUND: Dr. Cowings hopes to demonstrate that symptoms of so-called "space motion sickness" can be reduced by preflight autogenic feedback training (AFT). This technique has produced favorable responses in earth-based testing on human subjects.

The major emphasis for this investigation will be placed on preflight training for volitional control of autonomic responses. AFT is a combination of autogenic therapy and operant conditioning. Subjects are outfitted with bioinstrumentation packages which permit direct observation of physiological information (e.g. - heart rate).

Skylab and other U.S. and U.S.S.R. space missions have encountered an approximate 40 percent incidence of "space motion sickness." Symptoms are most frequent and most severe during the first 3-5 days of a mission. The impact of disabled crewmen on a short duration mission is critical with respect to their inability to perform normal payload operations.

PI OBJECTIVES: To determine the extent to which 0-g sickness in crewmen can be reduced by training them to control their own autonomic activity. To examine the relationships between physiological responses and the severity of 0-g sickness symptomatology in space. To develop predictive criteria for susceptibility to 0-g sickness.

PI HYPOTHESES: Preflight autonomic conditioning for suppression of motion sickness will enable crewmen to volitionally reduce the symptomatology of 0-g sickness. Physiological data recorded inflight in conjunction with a diagnostic scale can be used to identify the autonomic and behavioral characteristics of manifested symptoms. Preflight physiological data and visceral learning aptitude can predict individual differences in treatment effectiveness and susceptibility to 0-g sickness.

EXPERIMENT PLAN:

Preflight

Autonomic conditioning by the technique of autogenic feedback training (AFT) will be practiced by two crewmen as a method of suppressing 0-g motion sickness. Two other crewmen will serve as controls. The treatment group will receive 10 AFT sessions of 2.5 hours each at 10 day intervals beginning 120 days prior to launch. The crewmen will also self-administer AFT at home. All crewmen will receive baseline Coriolis motion sickness testing before AFT at 12 to 18 months prior to launch. There will be two repetitions of this test at 2 hours each. After AFT the treatment group will receive training tests (identical to baseline Coriolis motion sickness tests) where they will attempt to apply autonomic control to suppress motion sickness symptoms. The training tests will be 2 hours for each of four repetitions beginning 99 days prior to launch. Twenty-four to 48 hours prior to launch a final AFT verification session of 2 hours will be performed by the treatment group.

Inflight

All crewmen will self-administer a diagnostic scale at least once daily requiring approximately 5 minutes to report any motion sickness symptoms experienced. The treatment group will perform daily preventive AFT sessions of 15 minutes each. They will also perform counteractive AFT sessions at anytime that symptoms arise during the mission and thus cannot be timelined.

Postflight

All crewmen will attend a 2 hour debriefing within 14 days postflight.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The scientific objectives and the flight experiment protocol are the same now as they were in the original proposal. Between the initial proposal and the final Experiment Requirements Document (ERD), the requirements for formal autonomic conditioning training of flight crew members changed. Formerly the requirement was for one 7-day training session, no more than 30 days before launch. This was changed to ten 2.5 hour sessions, 10 days apart, during the 100 days preceding launch. Also added was the requirement for informal, daily, self-administered training sessions to be performed by the crewmembers. The reason for this was the PI's finding that a more distributed training schedule would be more effective than massed training. The ERD inflight measurements list mentions accelerator measurements of the subject's head and upper body movements. These measurements were not mentioned in the original proposal. The accelerometer measurements were added because reports of the biomedical results of Skylab indicated that 0-g sickness episodes were usually associated with head and upper body movements. The original proposal estimated that 4.5 to 9 hours of inflight crew time would be required. The ERD estimate is 13 hours. This increase is due, simply, to the PI taking a more detailed look at the requirement during the definition study.

SCIENCE: Dr. Cowings' investigation is designed to serve as a countermeasure to the relatively high incidence of space sickness which has occurred in previous spaceflights. This proposal is based on preflight autogenic-feedback training, a combination of autogenic therapy and operant conditioning, whereby subjects use biofeedback for volitional control of their autonomic responses. Since the impact of space sickness is most severe during the first few days in weightlessness, and since Shuttle missions are of relatively short duration, severe space sickness could critically restrict the ability of the crew to perform normal payload operations. This category 1 experiment thus directly addresses the first announcement of opportunity objective as well as overall payload and program objectives. Earth-based testing on human subjects shows that this approach has a reasonable likelihood of success.

This experiment is a potential source of conflict with the other human experiment recommended for the LS-1 mission (Young 072), but the exact nature of that conflict is undetermined at present. Basically, the conflict is caused by perturbation of the normal human response through the autogenic-feedback training. Then, other experiments on the vestibular system will find that their results apply to the altered state instead of the normal state. Since the autogenic-feedback training approach is relatively new, the actual effect of such training on other, more classical, vestibular studies has not yet been assessed. This must be done through the interaction of the principal investigators involved, and possibly through conduction of an auxiliary study. Because of the importance of the question addressed, and the unique approach that this experiment provides, it is essential to retain this experiment for inclusion in the LS-1 mission if the problems of scientific integration can be resolved. In any event, it is possible to incorporate this study on non-dedicated life science missions, as Spacelab is not required, and it is the intent to use this study in this manner where feasible.

EQUIPMENT: This investigation requires the LSLE cassette data recorder and a PI provided, body worn equipment consisting of transducer sets, electronics package, and numerical displays. The system is battery powered and has no Spacelab nor orbiter interface except stowage.

SUMMARY: This proposal deals with testing a countermeasure to an important operational problem - space motion sickness. It involves the use of "biofeedback" as an experimental modality. The uniqueness of the approach may interfere with other physiological studies recommended for LS-1, but the importance of the area to be studied makes this a high priority experiment. It is recommended that the experiment tentatively be selected for flight with the provision that scientific interference with other experiments, cost, and integration questions can be resolved.

FLOW CHART AND MEASUREMENT TABLE FOR EXPERIMENT #195 - COWINGS

TESTS*	Preflight				Launch	Inflight**						Recovery	Postflight
	L -540	L -120	L -99	L -1	L +0	L +1	L +2	L +3	L +4	L +5	L +6	R +14	
BASELINE CORIOLIS MOTION SICKNESS TEST 2 repetitions between (L-540) & (L-360)	4												
AMBULATORY BASELINE DATA COLLECTION 24 hours of data 2 repetitions between (L-540) & (L-360)	4												
AUTOGENIC-FEEDBACK TRAINING 10 repetitions between (L-120 & (L-10)		2											
SELF-ADMINISTERED AUTOGENIC FEEDBACK Performed by treatment group subjects at home 18 repetitions between (L-120) & (L-10)		2											
TRAINING TESTS 4 repetitions between (L-99) & (L-9)			2										
AUTOGENIC-FEEDBACK TRAINING VERIFICATION Performed once only				2									
SELF-ADMINISTERED DIAGNOSTIC SCALE Log symptoms experienced					4	4	4	4	4	4	4		
PREVENTIVE AUTOGENIC-FEEDBACK TRAINING					2	2	2	2	2	2	2		
COUNTERACTIVE AUTOGENIC-FEEDBACK TRAINING Performed when symptoms of zero-g sickness occur Control group subjects will perform the test without regulating the autonomic response levels					2	2	2	2	2	2	2		
DEBRIEFING													4

* All tests except DEBRIEFING will include measurements of electrocardiogram, heart rate, respiration rate, tidal volume, peripheral blood volume pulse amplitude, galvanic skin response, head movements, and reports of motion sickness symptoms (facial pallor, sweating, epigastric discomfort, etc.)

** Physiologic responses will be monitored continuously

PROPOSAL NUMBER: 781238

TITLE: A Study of the Effects of Space Travel on Mammalian Gravity Receptors

SPECIMEN: Rat

PRINCIPAL INVESTIGATOR: Ross, M.D., Ph.D.
AFFILIATION: University of Michigan, Ann Arbor, MI

CoIs/AFFILIATIONS:
None

BACKGROUND: Recent Russian studies (Cosmos 782) with rats indicate that, following prolonged weightlessness (15 days), ultrastructural and functional changes begin to take place in the vestibular apparatus of the inner ear, particularly the otolith organs. It is also postulated that degenerative processes occur in the otoconia (mineral crystals of the otolith) due to alterations in body fluids, hormonal balance, and electrolyte metabolism. There may be a relationship between degeneration in the otoconia and vestibular function in the development of space sickness. Edema of the tissue has been postulated (Vinnikov, 1968) to be a possible cause of otoconial degeneration or dissolution. Ultrastructural analyses will make it possible to study such changes in the vestibular organ.

The above objectives are based on the premise that otoconia-containing membranes (otoconial complexes) of the gravity receptors are dynamic mineral/organic material complexes existing in, and constantly interacting with a fluid medium, endolymph. It is postulated that prolonged exposure to the shifts in body fluids and to the changes in calcium, protein and carbohydrate metabolism that occur during spaceflight cause adverse effects upon the homeostatic processes in the inner ear that ordinarily preserve ion and fluid balance resulting in irreversible damage to the otoconial complexes. Concomitant or sequential changes are expected to occur in other related tissues.

PI OBJECTIVES: To determine the acute effects of exposure to spaceflight conditions on the biochemical and structural integrity of the otolith organs of the rat. To determine the extent to which such effects are chronic and/or progressive.

PI HYPOTHESES: Prolonged exposure to the shifts in body fluids and to the changes in calcium, protein and carbohydrate metabolism that occur during space flight cause adverse effects upon the homeostatic processes in the inner ear that ordinarily preserve ion and fluid balance resulting in irreversible damage to the otoconial complexes. Concomitant or sequential changes are expected to occur in other related tissues.

EXPERIMENT PLAN:

Male Wistar rats, weighing 250-300 grams, will be used. Five groups (I, II, III, IV, & V) of six animals each will be flown in the Spacelab and an additional five groups of matched subjects will provide one-g controls at the launch site.

Preflight

One group (I) of the animals will be injected with $^{45}Ca^{++}$ (4 mCi/kg body weight) one day prior to launch. Blood samples will be collected from the tail at 30 minutes, one, two, three, and four hours.

Inflight

Blood samples (50 ul) will be collected from seven animals (Group I and one animal from Group V) daily inflight. Centrifugation is optional, but refrigerated storage (4°C) of samples is mandatory. Group I will sacrificed before reentry and otoconial complexes will be removed and stored.

Postflight

The Groups II, III, and IV will be injected with $^{45}Ca^{++}$ postflight at 48 hours, one week and two weeks respectively. Blood samples will be obtained from these animals at 30 minutes, one, two, three, and four hours post-injection, at which time they will be sacrificed. Blood samples will be spun down and stored at 4°C for subsequent processing for liquid scintillation spectrometry.

Animals in Groups I through IV will be sacrificed by intracardiac perfusion with physiological saline while under ether anesthesia. Following decapitation, the temporal bones will be removed and placed in 70% alcohol, buffered to pH 7.4. A chip from the upper portion of the diaphysis of the femur will be collected. Microdissection of the otoconial complexes will be performed and the samples subjected to a series of washes in 7% alcohol (pH 7.4). Selected aliquots of the wash fluids will be analyzed for radioactivity. The saccular and utricular otoconial complexes will be processed separately. However, samples from all animals within each group will be pooled for liquid scintillation analysis. Otic bone chips and femur bone chips will be processed similarly for control counts of bone $^{45}Ca^{++}$ uptake.

Group V animals will be subdivided into three groups of two animals each to be sacrificed immediately upon recovery, and one and two weeks postflight respectively. The animals will be sacrificed by decapitation and the temporal structures dissected. Alternating left vs. right temporal structures will be designated for either scanning electron microscopy (SEM) or transmission electron microscopy (TEM) and processed accordingly.

Transmission electron microscopic analyses will focus upon the macular regions of the saccule and utricle with further emphasis upon the microvasculature for signs of edema.

Cochlear and ampullary regions will also be scanned for deleterious changes. The otoconia will be studied by scanning EM to determine any indications of altered calcium metabolism.

Ground Controls

Thirty matched animals will be maintained at the KSC Life Sciences Facility and they will be treated identically to the flight animals. Although a

simultaneous control is proposed, a delayed schedule would be advantageous in order to simulate flight conditions based upon the downlinked Research Animal Holding Facility (RAHF) data.

Measurements

Type	Units
Blood sample**	ul
Food consumption**	g/day
Water Consumption**	ml/day
Body weights	g
General activity levels**	counts
Blood $^{45}Ca^{++}$	pM/ug dry wt
Otic bone $^{45}Ca^{++}$	pM/ug dry wt
Femur bone $^{45}Ca^{++}$	pM/ug dry wt
Otoconial $^{45}Ca^{++}$	pM/ug dry wt
Saccular maculae - TEM	N/A
Utricular maculae - TEM	N/A
Vestibular ganglion - TEM	N/A
Cochlear-ampullary damage - TEM	N/A
Microvasculature - TEM	N/A
Tissue edema - TEM	N/A
Otoconial structure - SEM	N/A
Ambient temperature**	°C
Relative humidity**	%
Light and dark cycle**	h

* Data taken during flight procedures only.
** Data taken during flight and ground procedures.
All other data taken on the ground.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: There are two changes between the proposed experiment and the experiment as defined in the Experiment Requirements Document (ERD) - (1) inflight blood collection is planned from one additional animal, making a total of seven daily blood samples and (2) Group I animals are planned for sacrifice shortly before reentry instead of immediately after recovery.

SCIENCE: Valuable information will be obtained concerning the otoconial metabolism of calcium during and following exposure to spaceflight conditions. The establishment of a link between fluid/electrolyte metabolic alterations with vestibular changes will prove valuable in the ultimate determination of countermeasures for space motion sickness. The vestibular area of research is of high priority within NASA, and this experiment pertains to the second AO evaluation criterion. The results will directly aid in the interpretation of the human vestibular data and, because they require highly invasive techniques, could never be obtained from human subjects.

The investigator is very experienced in vestibular research and she is currently involved in other NASA projects. The techniques she proposes to use are standard ones that she uses routinely in her lab. Her experimental design is excellent and the chance of a successful mission is very high.

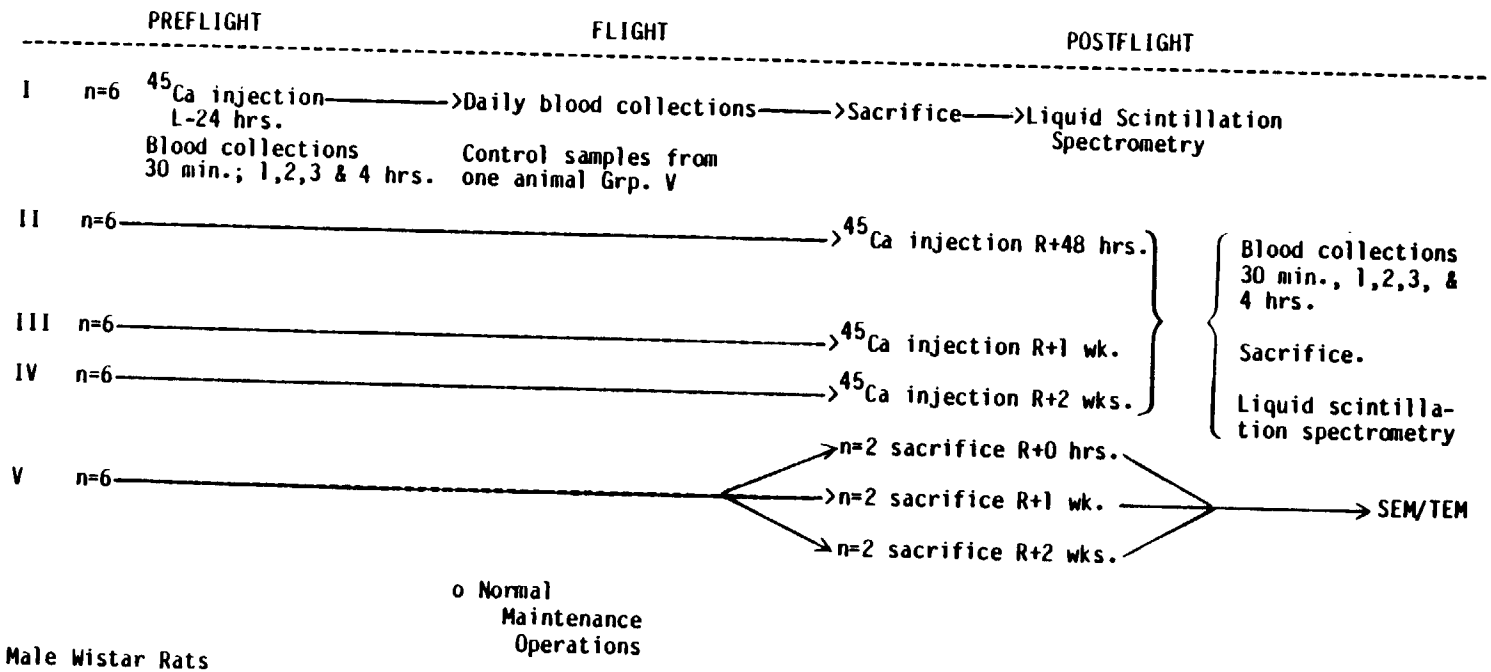
Besides being responsive to the AO criteria, this experiment meets the payload objectives and the program objectives. It possesses high merit, should be successful, employs the appropriate model, complements the human vestibular research, and addresses a fundamental biological problem.

In order to fit the experiment on the payload, the total sample size has been decreased from 30 rats to 24, and those must be shared with other investigators, but the science objectives have not been affected.

EQUIPMENT: The PI does not require any experiment unique hardware. She proposes to conduct a Spacelab vibration and noise simulation test as one of her supporting studies. The LSLE equipment to be used includes the RAHF, GPWS, refrigerator and voice recorder. Unique equipment will be a hematology kit, an Eppendorf microcentrifuge, and a rodent restraint device. The PI would like to evaluate the LSLE hematocrit centrifuge to determine if it will substitute for the Eppendorf centrifuge. Every effort will be made to guide her toward that decision.

SUMMARY: This experiment represents an opportunity to obtain information about the vestibular system that cannot be determined from human experimentation either aloft or on the ground. To demonstrate major morphological or biochemical alterations in the otolith apparatus in response to weightlessness would have considerable impact on the current theories of space motion sickness and is of major medical operational significance. The importance of the space motion sickness problem in humans strongly suggests that this experiment be included on the earliest possible payload. The slightly lower scientific priority of this proposal was significantly enhanced during definition when it was determined that inflight sacrifice could be technically integrated into the LS-1 hardware and timelines. Russian evidence of morphological damage may have been partially attributable to reentry. Inflight sacrifice eliminates this possibility and much strengthens the experimental design. It is recommended that this experiment tentatively be selected for flight on the understanding that inflight sacrifice will be provided but that fewer animals will be used.

EXPERIMENT FLOW DIAGRAM - ES 238



Male Wistar Rats
Total 30 Spaceflown
Total 30 Matched one-g controls

RENAL/ENDOCRINE

PROPOSAL NUMBER: 781192

TITLE: Fluid - Electrolyte Regulation During Spaceflight

SPECIMEN: Human

PRINCIPAL INVESTIGATOR: Leach, C. S., Ph.D.
AFFILIATION: NASA-JSC, Houston, TX

CoIs/AFFILIATIONS:
P. C. Rambaut, Sc.D./NASA-HQS, Washington, DC
W. N. Suki, M.D./Baylor College of Medicine, Houston, TX
P. C. Johnson, M.D./NASA-JSC, Houston, TX

BACKGROUND: Dr. Leach intends to document alterations in body fluid compartments, electrolytes, and renal or circulatory changes associated with acute exposure to weightlessness. These extensive measurements will extend the Skylab observations during the adaptive phase by examining specific mechanisms related to development of new homeostatic levels. The study is carefully planned to maintain adequate subject hydration to collect void-by-void urine volume and to perform constituent analyses postflight.

The scientific yield will be a detailed analyses of the acute renal response to weightlessness. These important data will help resolve numerous hypotheses which could not be evaluated during Skylab. It is expected that this study will determine the relative importance of several major pathways in correcting body fluid volume disturbances encountered during adaptation to weightlessness.

The specific contribution to LS-1 is a comprehensive analysis of renal endocrine responses which can be carefully integrated with the major cardiovascular experiments, together yielding a greater understanding of acute human response to weightlessness.

PI OBJECTIVES: To make detailed observations on the body fluid-electrolyte disturbances occurring in the first 24 hours of spaceflight. To extend Skylab observations in the adapting phase of spaceflight by examining specific hormonal, renal and circulatory mechanisms involved in maintaining homeostasis. To integrate the new information thus gained with Skylab findings in an effort to resolve some of the apparently contradictory renal function data collected during those missions.

PI HYPOTHESES: The loss of body water is complete by the third inflight day and is aggravated by motion sickness; body water may thereafter be maintained at a reduced level. These losses can be attributed largely to fluids lost from the legs. Loss of leg tissue fluid is opposed by fluid gain in upper body tissues. Plasma volume decreases about 500 ml early inflight and is sustained at the lower level. Factors other than aldosterone influence longer term sodium excretion, e.g., natriuretic factor, altered glomerular filtration rate and renal plasma flow, high plasma Ca++ levels, and prostaglandins. The primary controlling factor for antidiuretic hormone release is plasma osmolarity; antidiuretic hormonal effects on urine output may be counteracted by decreased prostaglandin synthesis or altered renal hemodynamics.

EXPERIMENT PLAN:

Preflight

The experiment design calls for four subjects to be involved for a 10 day ambulatory period preflight. Two additional control subjects will contribute daily urine specimens. All subjects will be on a fixed diet and encouraged to drink fluids in quantitites sufficient to maintain hydration.

Renal clearance tests, plasma volume, extracellular fluid studies, and total body water measurements will be performed on days L-10, L-5, L-1. Aldosterone secretory studies will be performed on days L-5 and L-1. All urine voids will be collected daily and frozen. Daily body mass measurements will be taken and should precede any blood draws. Arterial pressure, heart rate and central venous pressure shall be taken concurrent with renal clearance tests.

Subjects will void 2-4 hours prior to each renal clearance test and refrain from voiding until the test begins. Immediately prior to each study, an indwelling catheter will be placed in a superficial arm vein and will be used for drawing blood samples. A second venous catheter will be temporarily placed in the other arm for tracer injections. A 10.0 ml blood sample will be taken at the start of each study period and at the 90 minute mark on day L-1. Predetermined quantities of non-radioactive tracers (inulin and PAH) shall be injected into the second catheter of each subject. Time will be measured from completion of injection. Subsequently, 2.0 ml blood samples will be collected at 7, 20, 45, 60, and 90 minutes. Plasma will be separated and frozen.

Venous catheters used in the renal studies will be used for plasma volume and extracellular fluid studies. A 1.0 ml blood sample will be collected at the start of these studies. Two tracers (^{125}I-human serum albumin and ^{35}S-sulfate) will be administered in sequence. Blood samples (1.0 ml) will be collected at 30, 50, 90, and 120 minutes.

Total body water will be measured by the alcohol dilution technique at the same time the extracellular studies are performed. Each subject will ingest a premeasured ethanol dose. Expired air samples will be collected using the automatic sample loop of the breath alcohol analyzer at 90, 110, 120, 150, and 170 minutes post ingestion. The aldosterone secretory study will begin after the first early morning urine void. Ethanolic ^{3}H-aldosterone will be administered intravenously using the renal tracer catheter. Urine voids will be collected on a void-by-void basis for 24-hours following tracer ingestion.

Leg volume measurements will be made using the Life Sciences Laboratory Equipment (LSLE) limb volume measurement system on day L-5. Measurements will be made in 1-g after the subject has been supine for 30 minutes and again 30 minutes after the subject has assumed an erect posture.

Inflight

Renal clearance studies, aldosterone secretory studies, heart rate and arterial pressure and central venous pressure described preflight will be performed on Mission Day (MD) 1 (0-6 hrs inflight) and 6. Plasma volume and extracellular studies will occur on MD4 and 6. Body mass measurements, leg volume, and urine collection will occur daily.

In addition to the blood draw sequences listed under preflight, two 10.0 ml samples/subject will be required on MD1 (12 hours inflight) and at the start of MD2.

Postflight

On R+1 and R+7 all studies described under preflight shall be undertaken, an additional leg volume measurement will be taken on R+2. Body mass measurements and urine collections will occur daily.

Ground Studies

Five non-crew members shall undergo simultaneous testing with the crew during pre-, post-, and inflight phases.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The basic flight study program has been adhered to as originally proposed. Two additional supporting studies have been added: (1) aldosterone secretory study and (2) combination of renal and body fluid compartment studies. The purpose of these studies is to ensure multiple testing compatibility and the nature of tracer administration, as well as to reduce crew involvement time and minimize blood and urine sample number and volume required from the crew. These studies are well defined and seem appropriate in light of limited crew numbers and available crew time inflight. A breath analyzer has been added as an experiment unique piece of hardware to provde the PI with an accurate and easy to use instrument for breath alcohol analysis. Development of this off-the-shelf hardware item for flight precludes the necessity of modifying the LSLE trace gas analyzer, which as designed, cannot detect the ions under investigation by this PI.

SCIENCE: This experiment will address several important questions that are an outgrowth of Dr. Leach's Skylab studies and will gather data not previously available. In particlar, a comprehensive analysis will be performed of the acute and adaptive fluid-electrolyte responses to the headward shift of body fluids, as it effects renal, endocrine and circulatory function. Valuable measurements will include those related to renal plasma flows, body fluid compartments, central venous pressure, and plasma and urine biochemistry. The results will help validate several ground-based models of weightlessness and evaluate a complex set of hypotheses which accounts for the fluid and electrolyte losses in space flight. In addition, various elements of this experiment can be meaningfully integrated with other recommended flight experiments related to the erythropoiesis and cardiovascular systems (Dunn 261 and Blomqvist 294).

This category 1 experiment addresses the first announcement of opportunity objective as well as the primary LS-1 program and payload objectives. The definition phase demonstrated a high state of experiment readiness regarding hardware and protocol concepts with the assurance that all experiment objectives can be met. The use of several radioactive tracers is not expected to pose a significant problem. A series of important measurements early inflight will necessitate their collection in the middeck area immediately after entering weightlessness. This provision is currently acceptable. The

proposal, as submitted, would require a reduction in the number of replications toward the end of the mission. This should not, however, compromise the most valuable aspects of this experiment.

EQUIPMENT: This experiment requires 11 items of LSLE including the physiological monitoring system, strip chart recorder, urine monitoring system, large mass measurement device, rack mounted centrifuge, and inflight blood collection system. The P.I. will provide a breath alcohol analyzer and various kits including an aldosterone secretion study kit, a renal clearance kit, and a body fluid study kit. The LSLE items are planned LSLE either under development or in the procurment process.

SUMMARY: This experiment addresses the first AO objective as well as the primary LS-1 program objectives. The specific contribution of this experiment to LS-1 will be a comprehensive analysis of renal endocrine responses which can be carefully integrated with the major cardiovascular experiments, together yielding a greater understanding of the acute human response to weightlessness, particularly cephalic fluid shifts. The definition phase was successfully completed with assurance that the experiment will provide important results. Hardware and protocol concepts are well developed and indicate a high state of experimental readiness. It is recommended that this proposal be tentatively selected for flight on the understanding that the available crew time has been reduced, the budget is less than requested, samples must be shared, and the central venous pressure measurements will be performed by Blomqvist.

FLOW CHART AND MEASUREMENT TABLE FOR EXPERIMENT #192 - LEACH

TESTS	Preflight										Launch	Inflight								Recovery	Postflight						
	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	L +0H	L +6H	L +1D	L +2	L +3	L +4	L +5	L +6		R +1	R +2	R +3	R +4	R +5	R +6	R +7
RENAL CLEARANCE STUDY:																											
Renal plasma flow (PAH), GFR (inulin)	4					4				4	4							4		4							4
BLOOD SAMPLE:																											
Hematocrit Na^+, K^+, Ca^+, plasma proteins, creatine, ADH, angiotensin I, cortisol, ACTH, prostaglandins	4					4				4	4	4		4		4		4		4							4
URINE SAMPLE:																											
Na^+, K^+, Ca^+, Creatine, ADH, cortisol, prostaglandins	6	6	6	6	6	6	6	6	6	6	4	4	2	6	6	6	6	6		6	6	6	6	6	6	6	6
BODY FLUID VOLUMES:																											
extracellular fluid volume (^{35}S-sulfate), plasma volume (^{125}I-human serum albumin)						4										4		4		4							4
ALDOSTERONE SECRETORY STUDY (Ethanolic ^3H-aldosterone)						4				4	4							4		4							4
ARTERIAL BLOOD PRESSURE (cuff)	4					4				4	4					4		4		4							4
HEART RATE	4					4				4	4					4		4		4							4
BODY MASS MEASUREMENT	6	6	6	6	6	6	6	6	6	6	4		2	6	6	6	6	6		6	6	6	6	6	6	6	6
CENTRAL VENOUS PRESSURE (invasive arm catheter)	4					4				4	4							4		4							4
LEG VOLUME (Limb plethysmograph)						4					4	4		4		4		4		4	4						4
TOTAL BODY WATER (alcohol dilution)						4										4		4		4							4

PROPOSAL NUMBER: 781223

TITLE: Fluid and Electrolyte Homeostasis During Spaceflight: Elucidation of Mechanisms in a Primate

SPECIMEN: Squirrel Monkey

PRINCIPAL INVESTIGATOR: Moore-Ede, M. C., M.D., Ph.D.
AFFILIATION: Harvard Medical School, Boston, MA

CoIs/AFFILIATIONS:
C. Leach, Ph.D./NASA-JSC, Houston, TX
F. M. Sulzman, Ph.D./State University of New York, Binghamton, NY
C. A. Fuller, Ph.D./University of California, Riverside, CA

BACKGROUND: The changes in fluid and electrolyte homeostasis that occur during spaceflight are some of the most prominent physiological changes induced by weightlessness. Evidence from all past manned spaceflights suggests that one primary change was a shift of body fluids from the legs into the chest and head, symptomatically producing a rapid decrease in leg diameter, congestion in the thorax and head, and puffiness about the eyes. Ground-based studies with techniques such as saline infusion and water immersion indicate that a central blood volume increase leads to a decrease in antidiuretic hormone and aldosterone, a sodium-retaining hormone. These changes induce an increase in sodium and water excretion, which leads to a fall in plasma volume. Although these mechanisms are believed to account for the changes in fluids and electrolytes, direct data from Skylab studies in man have been limited in some respects. In particular, there are scant human data available for the first few hours and days of weightlessness, when most of the large shifts in fluid and electrolyte distribution occur. Additionally, the hormone, electrolyte, and fluid results were not entirely consistent with the proposed Skylab model. This study proposes to examine in small primates the mechanisms responsible for the adjustments in fluid and electrolyte homeostasis that promptly occur in weightlessness.

PI OBJECTIVES: To determine whether the monkey's cardiovascular, renin, angiotensin, aldosterone, renal fluid and electrolyte responses are similar to those of man.

To confirm a ground-based model of fluid shifts during weightlessness and its applicability to humans in spaceflight.

To gather baseline information for future studies of the mechanisms and consequences of fluid and electrolyte shifts.

PI HYPOTHESES: Weightlessness will induce an increase in central venous pressure and a subsequent diuresis and natriuresis which will be maintained throughout the flight phase.

In response to initiation of weightlessness, there will be a transitory kaliuresis and a fall in plasma potassium concentration. The hypokalemia will be maintained throughout the mission, but will recover rapidly after landing.

The hypokalemia will induce detectable changes in the electrocardiogram.

There will be an initial fall in plasma aldosterone on assumption of weightlessness but this will recover to baseline or above baseline levels by the second day of the mission.

EXPERIMENT PLAN:

Preflight

Twelve male squirrel monkeys will be trained to sit in restraint chairs, implanted with arterial and venous catheters (in the iliac vessels), and studied in control experiments to acclimatize them to the flight protocol sequence, beginning approximately 3 months before flight. In these studies, urine and feces are collected from the animals; blood samples are drawn from the arterial catheters; and continuous tracings of arterial blood pressure, ECG, heart rate and central venous pressure are obtained. The experiments are conducted with the monkeys sitting in chairs, pressing levers for food reward and maintained in a 12:12 hour light-dark (LD) cycle. Constituents of the plasma, urine and feces will be analyzed. These control studies will obtain both baseline data for comparisons with the inflight experiment and accustom the monkeys to the details of the protocol and handling. After these experiments are completed, 4 animals will be selected for the flight experiment, the remainder of the animals being used as back-up support.

Inflight

Four restrained monkeys will be housed in individual light-tight isolation Research Animal Holding Facility (RAHF) cages. Each animal will be provided with a LD 12:12 hour cycle with lights-on at 60 lux, and lights-off at less than 1 lux. The time of lights-on each day will be scheduled for 2 hours before the planned launch time (0500 hours). Individual variables that will be monitored include gross motor activity (by an infrared or similar device), feeding and drinking activity (by RAHF sensing devices), arterial blood pressure and heart rate (from sensors attached to the arterial catheter), central venous pressure (from a pressure transducer attached to the venous catheter), the ECG (from a modified V-4 electrocardiographic lead placement of skin electrodes), blood samples drawn intermittently throughout the mission from the arterial catheter, and urine samples collected in 4 hourly aliquots throughout the experiment. The blood plasma will be separated by centrifugation and frozen for postflight analysis. Blood cells, mixed with saline, will be reinfused by the venous catheter to maintain an isovolumic monkey preparation. The urine samples will be refrigerated for postflight analysis. Fecal material will be saved each day from the RAHF collection devices and stored in individual daily packages for subsequent analysis.

Postflight

Animals will be returned to the investigator's laboratory for a series of postflight studies which will undertake ground-based simulations of the light-dark cycles, the launch profile using the Lower Body Positive Pressure (LBPP) simulation, and the thermal conditions of the flight. All specimens collected inflight and in the control and postflight studies will be analyzed

as follows: blood plasma samples for aldosterone, cortisol, renin, arginine-vasopressin, potassium, and sodium; urine samples for potassium, sodium, chloride, osmolality, aldosterone, cortisol, creatinine, calcium, phosphate, and arginine-vasopressin; fecal samples for potassium, sodium and phosphate, calcium and total nitrogen. Comparisons will be undertaken with these data and with the data from arterial blood pressure, venous blood pressure, ECG, and heart rate during these experiments.

Ground Control

A ground control study using a separate group of animals will be performed in Boston before and after the flight. This group will be compared with the flight group. Preflight and postflight studies of the flight group will take place in Boston.

Measurements

Type	Unit
Weight**	kg
Hematocrit	%
Catheter function**	N/A
Catheter flora	Colonies
Pooled urine (4 hours)**	ml/4h
Water consumed (4 hours)**	ml/4h
Food pellets consumed (4 hours)**	g/4h
Arterial blood presure**	Torr
Venous blood pressure**	Torr
Heart rate**	beats/min
Plasma sample volume**	ml
Plasma aldosterone	pg/ml
Plasma cortisol	mg/ml
Plasma renin activity	ng/ml/h
Plasma arginine-vasopressin	pg/ml
Plasma potassium	mEq/l
Plasma sodium	mEq/l
Urine potassium	uEq/h
Urine sodium	uEq/h
Urine chloride	uEq/h
Urine osmolarity	mOsm/kg
Urine aldosterone	pg/h
Urine cortisol	ug/h
Urine creatinine	mg/h
Urine calcium	uEq/h
Urine phosphate	uEq/h
Urine arginine-vasopressin	pg/h
Feces**	gm/24h
Fecal potassium	mEq/24h
Fecal sodium	mEq/24h
Fecal phosphate	mEq/24h
Fecal calcium	mEq/24h
Fecal total nitrogen	g/24h
Calibration pressures	Torr
ECG**	mV

Type	Unit
Ambient temperature**	°C
Atmospheric carbon dioxide*	Torr
Atmospheric oxygen*	Torr
Atmospheric water*	Torr
Barometric pressure*	Torr
Noise**	dB
Light cycle**	h
Light intensity**	Lux

* Data taken during flight procedures only.
** Data taken during flight and ground procedures.
All other data taken on the ground.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The proposal provided flexibility and alternatives to suit the program as the program matured. Considerable effort has been expended in addressing the technical challenge posed by this experiment. The major problems have been solved and the experiment now has a good likelihood of success. The Experiment Requirements Document (ERD) and the proposal have few differences. Some of the requirement changes are:

1) a reduction of light intensity from 600 lux to 60 lux;

2) elimination of 75 dB white noise in the animal flight cages;

3) elimination of gross motor activity monitoring;

4) addition of urine and plasma arginine-vasopressin measurements;

5) addition of fecal analyses;

6) addition of ECG.

The proposed population of 6 ± 2 animals was limited to 4 animals. Three of the original six animals were to have plasma samples withdrawn at 4-hour intervals on L + 1 days and R - 2 days. All animals now yield 4-hour plasma samples on L + 1 day and single plasma samples on subsequent flight days.

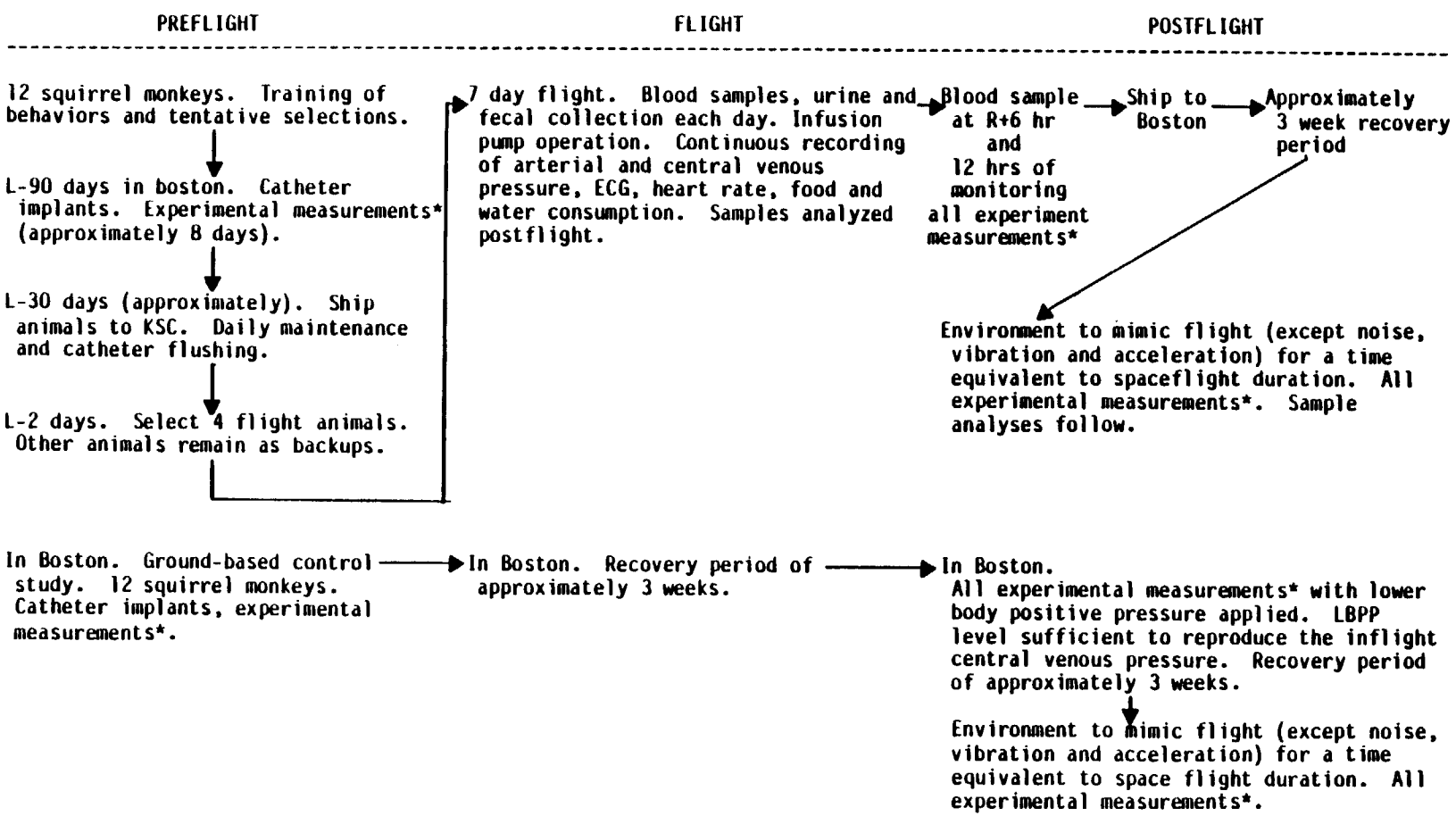
SCIENCE: The hypotheses are sound and the experiment is well planned and thought out. The ambitious experimental design includes excellent control studies. Although the experimental group size is less than optimal, the frequent sampling interval will provide a large number of data points, thus reducing statistical variability. The squirrel monkey is a good model for studies of renal and fluid/electrolyte shifts in weightlessness. These animals have been shown to develop motion sickness which may provide some risk to this restrained primate. This experiment directly supports the AO, program and payload objectives in that it addresses fluid shifts, one of the most often observed alterations in the physiology of the crews. Due to the very ambitious nature of the original proposal, some minor approaches may

have to be compromised. The original proposal suggested that a dedicated squirrel monkey holding facilty would be developed for this experiment. During the definition phase activity, it was concluded that the objectives of the experiment could be carried out with only slight modifications to the current squirrel monkey RAHF design. In order to finalize the selection of this experiment, the investigator will have to agree to this small change in animal housing design and also to agree to accommodate portions of another squirrel monkey experiment (781039, Fuller).

EQUIPMENT: Experiment unique equipment includes restraint systems (4), automatic urine collectors (4), two-channel infusion pumps (4), pressure transducers and signal conditioners (8), heart rate units (4) probably BTS, hematology kit, temperature transducers (BTS), light intensity monitor and a portable cooler.

SUMMARY: The changes in fluid and electrolyte homeostasis that occur during spaceflight are among the most prominent physiological changes induced by weightlessness. This investigation will explore these changes in a comprehensive fashion; it will also seek to establish the squirrel monkey as a human surrogate in this area. Considerable engineering effort and some modifications were developed during the definition phase which have alleviated early concerns about hardware development. The experiment now has an excellent likelihood of success. It is recommended that this investigation tentatively be selected for flight on the understanding that it be combined with Fuller (781039), that technical changes which provide a greater focus on medical problems be accepted, and that fewer monkeys be used.

EXPERIMENT FLOW DIAGRAM - ES 223



* Experiment measurements consist of:
Food and water consumption, urine volume and constituents, fecal constituents and mass, arterial pressure, central venous pressure, ECG and heart rate.

PROPOSAL NUMBER: 781039

TITLE: Thermoregulation in Primates in the Space Environment

SPECIMEN: Squirrel Monkey

PRINCIPAL INVESTIGATOR: Fuller, C.A., Ph.D.
AFFILIATION: University of California, Riverside, CA

CoIs/AFFILIATIONS:
M. C. Moore-Ede, M.D., Ph.D./Harvard Medical School, Boston, MA
F. M. Sulzman, Ph.D./State University of New York, Binghamton, NY

BACKGROUND: The homeothermic animal is able to regulate body temperature by controlling heat production and heat loss. These thermoregulatory mechanisms achieve a heat balance within the organism that is modified by both intrinsic (endocrine, cardiovascular and metabolic) and extrinsic (environmental) factors. As a result, body temperature is not constant, but exhibits a regular 24-hour sinusoidal rhythm (a circadian rhythm) about a mean temperature. Several of the extrinsic factors such as noise, lighting intensity, vibration or acceleration can shift the period of the normal biorhythm so that it becomes longer or shorter.

Numerous thermoregulatory changes occur when monkeys are exposed to hypergravity produced by centrifugation. These changes include decreased colonic temperature and skin temperatures accompanied by a decreased oxygen consumption. Such observations support the view that a decreased heat production and an increased heat loss are the thermoregulatory responses involved.

Spaceflight experiences with animals have also suggested a decreased body temperature. During the Biosatellite 3 flight, the primate showed a gradual decline in body temperature and the period of the temperature rhythm increased despite a constant light/dark cycle. Evidence indicates that a primate's temperature rhythm can be entrained to a constant ambient light/dark cycle. Yet, in the case of Biosatellite 3, the data suggests that the circadian rhythm became free running and was no longer entrained to the ambient light/dark cycle.

Thus, the input signals for temperature regulation could be influenced by the extrinsic factors produced by spaceflight.

PI OBJECTIVES: To characterize the physiological regulation of body temperature during spaceflight. To determine if the expressions (temperature, feeding or drinking) of the circadian timekeeping system are influenced by lift-off, reentry, or weightlessness. To determine if the circadian timekeeping system maintains internal synchrony among variables and external synchrony with the environment.

PI HYPOTHESES: Body temperature will be regulated at an abnormal level during spaceflight. The 24-hour means, amplitudes and waveforms of circadian rhythms will be modified by spaceflight. All circadian rhythms will not remain synchronized with the 24-hour light-dark cycle during spaceflight.

EXPERIMENT PLAN:

In this experiment, core temperature, 5 skin temperatures, ambient temperature, and food and water consumption will be monitored continuously on 4 monkeys. This mission will specifically address the control of thermoregulation in spaceflight, 24-hour entrainment and decoupling of the circadian timekeeping system.

Preflight

Squirrel monkeys will be trained to sit in chairs, press levers for a food reward, and establish circadian rhythyms on an L:D 12:12 hour light cycle. Baseline data for each intended inflight measurement will be obtained.

Inflight

Four monkeys will be individually placed in visually isolated Research Animal Holding Facility (RAHF) cages.

During flight, the following parameters will be measured: skin temperature at five sites, colonic temperature, ambient temperature, feeding activity and drinking activity. These parameters will be measured periodically throughout the light and dark phases of each flight day. The information will be digitized and stored for postflight analysis.

Individual feeding and drinking activity will also be monitored by the RAHF sensing devices. The trained animals will press a lever repetitively to obtain the food pellets. The pellet dispenser will operate on a fixed ratio of lever presses for each pellet.

Each of the animals will be entrained to an L:D 12:12 hour light-dark cycle. Maximum and minimum light intensities have been specified at 60 lux and less than one lux respectively.

Monitoring thermistor devices will be used to detect peripheral and deep body temperatures. These thermistors will be affixed to five skin sites (proximal and distal tail, foot, abdomen, thigh, and calf) and one thermistor will be inserted 6 cm past the anus for colonic temperature. A switching device will sequentially sample each thermistor (TBD rate and interval) and the information will be digitized and collected on a storage device (TBD). The collected data can be recombined into a weighted formula that indicates body temperature or a temperature profile for any measured body site.

Postflight

The animals will be monitored on the ground for up to 96 additional hours to observe recovery responses. Five months after the flight, the animals will be reexamined for postflight recovery steady-state values.

Ground Control

A seven day ground control study will take place in the investigator's laboratory about five months postflight. The animals will be housed in a "RAHF like" environment. All environmental conditions occurring during the

spaceflight (except launch effects and weightlessness) will be duplicated. The experimental measurements will be taken as in the actual flight experiment.

Measurements

The measurements are for each animal.

Type	Unit
Food pellets consumed**	Pellets/0.5h
Water consumed**	ml/0.5h
Colonic temperature**	°C
Skin temperature I**	°C
Skin temperature II**	°C
Skin temperature III**	°C
Skin temperature IV**	°C
Skin temperature V**	°C
Animal weight	g
Animal health**	N/A
Temperature probe calibrations	N/A
Water bath temperature	°C
Ambient temperature**	°C
Each cage temperature*	°C
Each cage light cycle**	h
Visual isolation**	N/A
Barometric pressure**	Torr
Atmospheric oxygen*	Torr
Atmospheric carbon dioxide*	Torr
Noise level**	dB
Cage lighting level**	Lux

* Data taken during flight procedures only.
** Data taken during flight and ground procedures.
All other data taken on the ground.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The Experiment Requirements Document (ERD) deleted several experiment conditions or procedures from the proposal. The deleted items are:

1) no measurement of oxygen, carbon dioxide or barometric pressure during Spacelab activities;

2) no provision for 75 dB of white noise in the animal flight cages;

3) no monitoring of gross motor activity.

The proposed population of 6 $\pm$ 2 animals was limited to 4 animals. These original 6 animals were divided into two groups that were 180 degrees out of phase with respect to the light/dark cycle. Animals are now exposed to the

same light/dark cycle and the intensity requirement was reduced from 600 lux to 60 lux.

Skin temperature sensor locations were identified in the proposal but are not specified in the ERD.

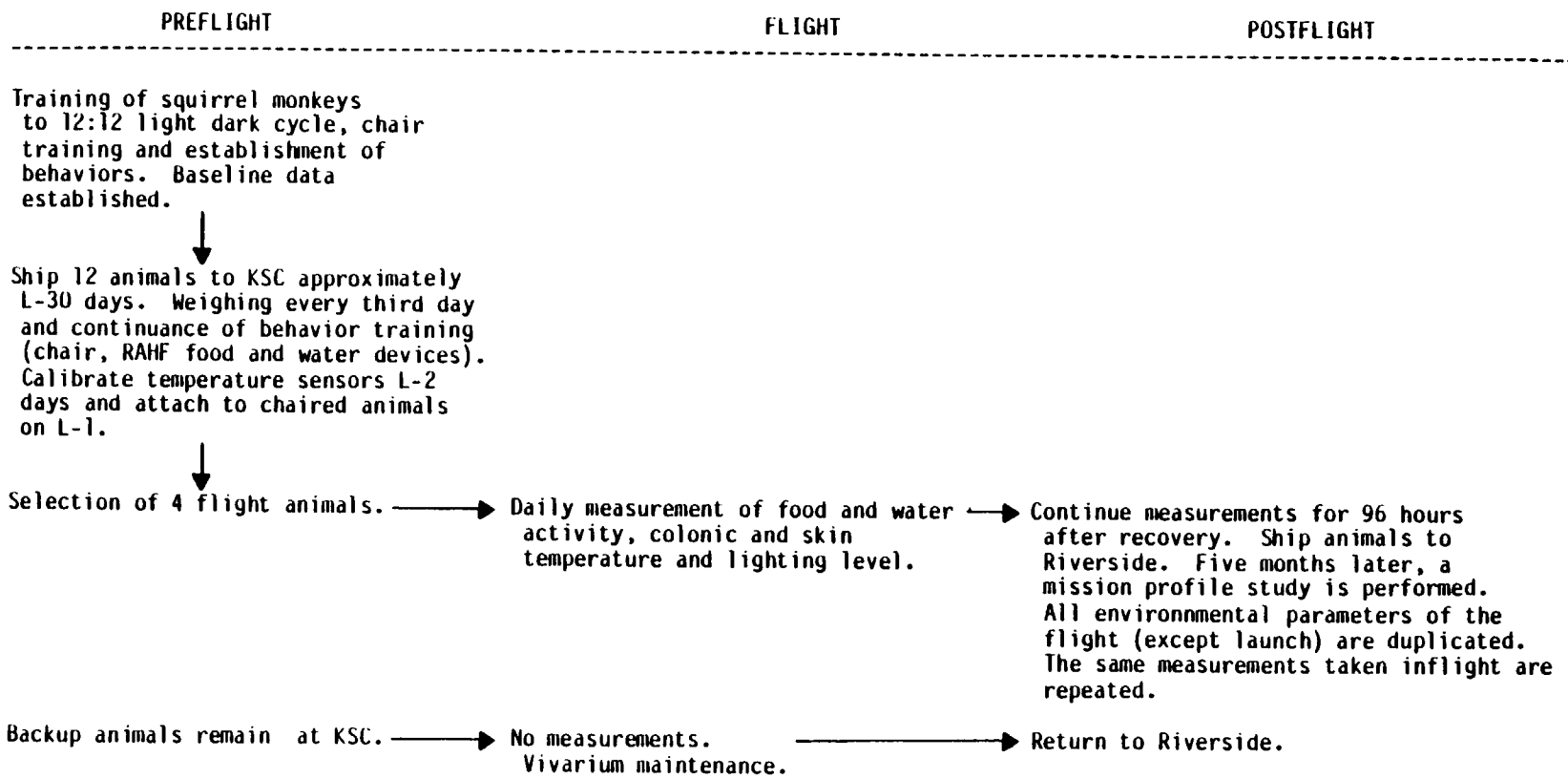
The events of the definition phase indicate that the concerns about hardware design which led to the original categorization are no longer a problem. The utilization of the current squirrel monkey RAHF is the key to the success of this experiment. Data from the biological telemetry system should be adequate to determine the end-points requested by the investigator.

SCIENCE: Circadian periodicity of temperature rhythms was addressed on the Biosatellite III primate flight, but the results were inconclusive. This experiment is designed to provide a proper test of the effects of spaceflight on body temperature periodicity in squirrel monkeys. The experimental paradigm is good, and the scientific yield will be positive. Although the experimental group size is less than optimal, the frequent sampling interval will provide a large number of data points, thus reducing statistical variability. A large experimental population will increase the experimental confidence level.

EQUIPMENT: The development of a suitable Zero-g restraint-waste management system will require engineering design support that must come from an interaction of the investigators and the LSFEP. Experiment unique equipment for this experiment is as follows; 4 restraint chairs; analog recorder; temperature probes, temperature probes, light intensity monitors; and signal conditioners. In addition some LSLE is required: the squirrel monkey RAHF; microcomputer; and the DEMS.

SUMMARY: Definition revealed that the PI's ground-based research has solved several technical problems responsible for the original categorization. This experiment will provide conclusive data concerning the resetting of body temperature in weightlessness. This protocol can easily be incorporated into the investigation of Moore-Ede (781223) and the ability to conduct these two investigations simultaneously makes this an efficient and scientifically powerful combination. It is recommended that this investigation tentatively be selected for flight on the understanding that it be combined with Moore-Ede, that several simplifying technical changes be accepted, and that fewer animals be used.

EXPERIMENT FLOW DIAGRAM - ES 039



HEMATOLOGY

PROPOSAL NUMBER: 781261

TITLE: Influence of Space Flight on Erythrokinetics in Man

SPECIMEN: Human

PRINCIPAL INVESTIGATOR: Dunn, C. D. R., Ph.D.
AFFILIATION: Northrop Services Incorporated, Houston, TX

CoIs/AFFILIATIONS:
P. C. Johnson, M.D./NASA-JSC, Houston, TX

BACKGROUND: Dr. Dunn proposes to extend planned SL-1 human erythropoietic studies. Specific erythropoietic control factors would be assessed during the acute exposure to weightlessness. This experiment is complemented by LS-1 animal research utilizing a rat model of the human erythropoietic system.

The general approach requires venipunctures for blood sampling and inflight injections of radioisotopes to determine blood volume, estimates of erythropoietin activity, and the analysis of serum components related to erythropoiesis and iron kinetics.

The specific yield from this experiment will be a description of erythrokinetics during the acute exposure to weightlessness. Data should determine whether red cell mass loss is due to decreased production or increased hemolysis. These results will amplify the knowledge gained during Skylab where no radioisotope measurements were performed inflight. These data support the Announcement of Opportunity (AO) criteria to investigate known physiological changes consequent to weightless exposure in man.

PI OBJECTIVES: A. To investigate the role of erythropoietin and erythropoietin inhibitors in the reduction of circulating erythrocyte mass during spaceflight. B. To investigate the possible roles of ineffective erythropoiesis and hemolysis in the reduction of red cell mass as observed in spaceflight.

PI HYPOTHESES: Spaceflight-associated reduction of erythrocyte mass is due to erythropoietin-related suppression of normal erythropoiesis. Spaceflight-associated reduction of erythrocyte mass is due to increased hemolysis and subsequent inhibition of erythropoiesis.

EXPERIMENT PLAN:

Preflight

Five crew members will participate in preflight studies beginning on L-45. A 20.0 ml blood sample will be collected from each crew member. Following the initial blood draw, 12.5 ml of blood will be collected into 2.5 ml of anticoagulant. This blood will be tagged with $Na_2{}^{51}CrO_4$, (37.5uCi), mixed for 4 minutes and then 50 mg ascorbic acid will be injected into the bag to halt further uptake by the cells. While this tagging procedure is being carried out, 1.0 ml radioiodinated human serum albumin (HSA) (2.0uCi ^{125}I) will be injected into each subject. Ten milliliters of the tagged suspension

will then be immediately injected into each subject. After 30 minutes, 5.0 ml blood will be collected followed by an injection of ^{59}Fe-ferrous citrate (1.0 ml, 2.0uCi ^{59}Fe).

On day L-30 and L-5, 20 ml blood will be collected from each crew member and analyzed postflight. On L-15 the L-45 day procedures will be exactly replicated.

Inflight

On Mission Day (MD) 2, a 20.0 ml blood sample will be collected from each crew member via a butterfly infusion catheter. 100 ul of this blood will be taken up in a heparinized hematocrit tube and stored at 4°C for postflight analysis. The remaining blood sample will be centrifuged and frozen for return analysis.

On MD 3, a 20 ml blood sample will be collected, the hematocrit collected and measured and a second heparin filled hematocrit tube prepared as on MD2. Immediately following the blood draw, 1.0 ml radioiodinated HSA will be injected into each crew member. After 30 minutes, a 5.0 ml blood sample will be collected followed by an injection of 1.0 ml ^{59}Fe-ferrous citrate (2.0uCi). Hematocrit determinations will be performed and the samples will be centrifuged and frozen for return analysis.

On MD 4 and 7, the procedures outlined for MD 2 will be repeated for each crew member.

Postflight

On R+0 and R+14, preflight procedures of L-45 will be exactly replicated. On R+1, 2, 7; 20.0 ml blood samples will be collected from each crew member, centrifuged and frozen for analysis.

Ground Studies

Pre-, post-, and inflight procedures executed on the crew will be exactly replicated on a group of carefully selected volunteers. The timeline will duplicate the crew timeline.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: As a result of definition studies the preflight experiment sessions decreased from 7 to 4; inflight sessions decreased by 2; and postflight sessions decreased by 4 from the original proposal. The CoI has changed affiliations and is now employed as a support contractor at JSC. Therefore, all facilities, including supporting personnel is located at JSC.

SCIENCE: This important category 1 study will seek factors responsible for the loss of red cell mass which has been consistently observed in spaceflight crews. As such, this investigation supports the announcement of opportunity, program, and payload objectives with regard to examination of known human physiological changes which may affect human performance. The results will not only provide valuable erythrokinetic and blood volume data, previously unavailable inflight, but will also complement other recommended studies

(Dunn 012 and Johnson 141) by verifying the rat as a zero-g model for human erythropoiesis. No aspect of this experiment requires alteration, but combination of inflight procedures (i.e., tracer injection and blood draws) with other recommended experiments (Blomqvist 294 and Leach 192) will be essential to reduce blood volume samples and to conserve crew time allotment.

This experiment requires radioisotope injections and, although this has not previously been performed inflight, it is the current view that these procedures can be safely accomplished. Although the decrements in red cell mass during a one-week flight will not be large, it is believed that the underlying erythrokinetic changes should be easily measurable by the sensitive technique employed. Therefore, all experimental objectives should be successfully achieved.

EQUIPMENT: This investigation requires several items of LSLE hardware including the inflight blood collection system, the rack-mounted centrifuge, the freezer, refrigerator and the hematocrit centrifuge. All are planned LSLE items either under development or planned as competitive procurements. No investigator supplied equipment is necessary for this experiment.

SUMMARY: This experiment is specifically concerned with the decrease in red cell mass that previous space flight crews have consistently exhibited. This experiment seeks to determine whether reduced red cell mass in humans is due to decreased production or increased hemolysis. It is complemented by recommended animal research that involves parallel studies (Johnson 781141 and Dunn 781012). Definition phase activities indicate this experiment can be successfully implemented. It is recommended that this investigation tentatively be selected for flight on the understanding that specimens be shared and a modest descoping be accepted along with related reductions in budget and crew time.

FLOW CHART AND MEASUREMENT TABLE FOR EXPERIMENT #261 - DUNN

TESTS	Preflight				Launch	Inflight				Recovery	Postflight				
	L -45	L -30	L -15	L -5		L +1	L +2	L +3	L +6		R +0	R +1	R +2	R +7	R +14
BASIC HEMATOLOGY MEASUREMENTS: RBC count, Hemoglobin, indices (MCV, MCH, MCHC), reticulocyte count, 2-3 DPG, ATP	5	5	5	5		5	5	5	5		5	5	5	5	5
BASIC SERUM CHEMISTRY: total protein, protein distribution, haptoglobin, bilirubin, transferrin, ferritin, Na^+, K^+, osmolality, iron	5	5	5	5		5	5	5	5		5	5	5	5	5
HEMATOCRIT (microcentrifugation)	5	5	5				5				5	5	5	5	5
HEMOGLOBIN-OXYGEN P_{50}	5	5	5	5		5	5	5	5		5	5	5	5	5
RED CELL MASS AND RBC SURVIVAL (^{51}Cr tagging of RBC's)	5		5				None				5				5
PLASMA VOLUME (^{125}I-radioiodated human serum albumin)	5		5				5				5				5
RBC PRODUCTION RATE (^{59}Fe-ferrous citrate injection)	5		5				5				5				5
ERYTHROPOIESIS: Erythropoietin, erythropoietin inhibitors	5	5	5	5		5	5	5	5		5	5	5	5	5

PROPOSAL NUMBER: 781141

TITLE: Regulation of Blood Volume During Spaceflight

SPECIMEN: Rat

PRINCIPAL INVESTIGATOR: Johnson, P.C., M.D.
AFFILIATION: NASA-JSC, Houston, TX

CoIs/AFFILIATIONS: None

BACKGROUND: Gemini, Apollo, and Skylab astronauts have shown decreases in their red blood cell (RBC) mass of approximately 10%. The mechanism of this loss is unclear. Data from Gemini VII suggested the decrease to be related to a decreased life span of the cells, but the Apollo flight data have failed to support this. Data from these flights suggest a decrease in the production of RBC's. The decreased mass was initially attributed to the hyperoxic environment of the Gemini and Apollo capsules. Skylab, however, did not have the hyperoxic environment and the crew members of each Skylab mission still had an average of 10% reduction in RBC mass. The mean RBC life span was normal. Approximately one week was needed for regeneration of RBC's to occur.

Plasma volumes were shown to decrease in Skylab crew members. The plasma changes are similar to those seen with the fluid shifts accompanying prolonged bed rest. Such changes would be expected, as the plasma pool in humans compensates for gravity effects with postural shifts.

Cosmos 782 and 936 data (Leon, et al.) suggest that in 0-g, the rat undergoes similar changes to man in RBC mass and plasma volume, as evidenced by decreased bone marrow and spleen RBC precursors.

PI OBJECTIVES: To determine if the effect of the spaceflight environment on the regulation of blood volume in the rat is comparable with that in man. To verify the rat as a suitable experimental animal to further elucidate the mechanisms of the documented changes in man.

PI HYPOTHESES: The erythropoietic system of the rat will be suppressed during weightlessness. The magnitude of this suppression will be such that a measurable decrease in the rat red blood cell mass will occur. Weightlessness may cause a decrease in the rat's plasma volume.

EXPERIMENT PLAN:

Preflight

Fifty-two adult female rats will be weight-stabilized on the flight diet for 6-8 weeks prior to flight. Daily weighings of the rats will be made and the amount of available food will be adjusted to maintain the animals' weights at approximately 300 g.

At approximately L-30, twenty-one animals will be selected for flight. They will be divided into 3 groups of 7. Groups 1 and 2 will be subjected to measurements for red cell mass, plasma volume, hematocrit, reticulocyte count, reticulocyte classification and red blood cell morphology (Measurements A).

At L-7, these measurements will be made for Group 3. Additionally, Group 3 will have blood samples withdrawn on L-9, L-7, and L-5 for the determination of ^{51}Cr survival time (Measurement B).

Inflight

Inflight activities are limited to body mass measurements for Group 1 animals days L+3 and L+6.

Postflight

On day R+0, Group 1 will be placed in an hypoxic chamber for 3 days. Group 2 will undergo Measurements A again. Group 3 will be injected with ^{59}Fe.

Days R+1, R+3, and R+5 Group 3 will be bled and measurements of ^{59}Fe RBC incorporation, ^{51}Cr survival time, haptoglobin, transferrin, ferritin, hemoglobin, and total protein (Measurements D) will be made.

At R+10 all three groups of animals will be bled for Measurements A again.

Ground Control

A ground control experiment will be performed at KSC. The control animals will be subjected to the above protocol ten days later than the flight animals. This delay for the control animals will continue through the entire experiment.

Measurements

Type	Units
Body mass**	g
Red blood cell mass	ml
Plasma volume	ml
Hematocrit	%
Reticulocyte count	%
Reticulocyte age classification	N/A
Red blood cell morphology	N/A
^{59}Fe RBC incorporation	%
^{51}Cr survival time	days
Haptoglobin	mg/dl
Transferrin	mg/dl
Ferritin	mg/dl
Total protein	g/dl
Hemoglobin	g/dl
Foodconsumed**	g/day
Water consumed**	ml/day
Ambient temperature**	°C
Ambient relative humidity**	%
Light-dark cycle**	h
pO_2 of hypoxic chamber	mmHg

* Data taken during flight procedures only.
** Data taken during flight and ground procedures.
All other data taken on the ground.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The proposal described three groups of 7 to 10 rats, each subjected to a series of blood measurements preflight and postflight. One of these groups was subjected to hypoxia in-flight, the second group was subjected to hypoxia postflight, and the third group was not subjected to hypoxia.

The ERD reflects two major changes. The inflight hypoxic treatment was deleted due to cost considerations. The postflight hypoxia treatment for 7 rats remained. Due to the amount of blood required to do the proposed measurements pre- and postflight, the remaining 14 animals have been divided into two groups. Each group will be used for part of the measurements. This modification was necessary to preclude experiment associated blood losses from stimulating erythropoiesis.

Additional ground-based data are required to establish the specificity and sensitivity of the erythropoietin assay (i.e., the availability of the specific antisera for the proposed immunodiffusion techniques for the immunologically compatible serum protein).

SCIENCE: This study is designed to validate the rat as a model for red cell mass changes associated with spaceflight. Based on head-down restraint studies showing that the rat undergoes a fluid shift, these investigators predict that the rat will be a good model for hematological changes in spaceflight. The astronauts' red cell mass decreases are suspected to be related to fluid shifts. The experiment does not yield any information on the hematological changes which occur during spaceflight, since the investigators do not request blood samples in flight. A strong argument could be made to attempt to measure inflight blood samples in order to measure the processes controlling the red cell mass decrease free of the complications of reentry stress. This experiment is a natural combination with another hematology experiment (781012, Dunn), and efforts would be made to effect this combination. If the experiment is selected, the investigator should be agreeable to this suggested combination of effort.

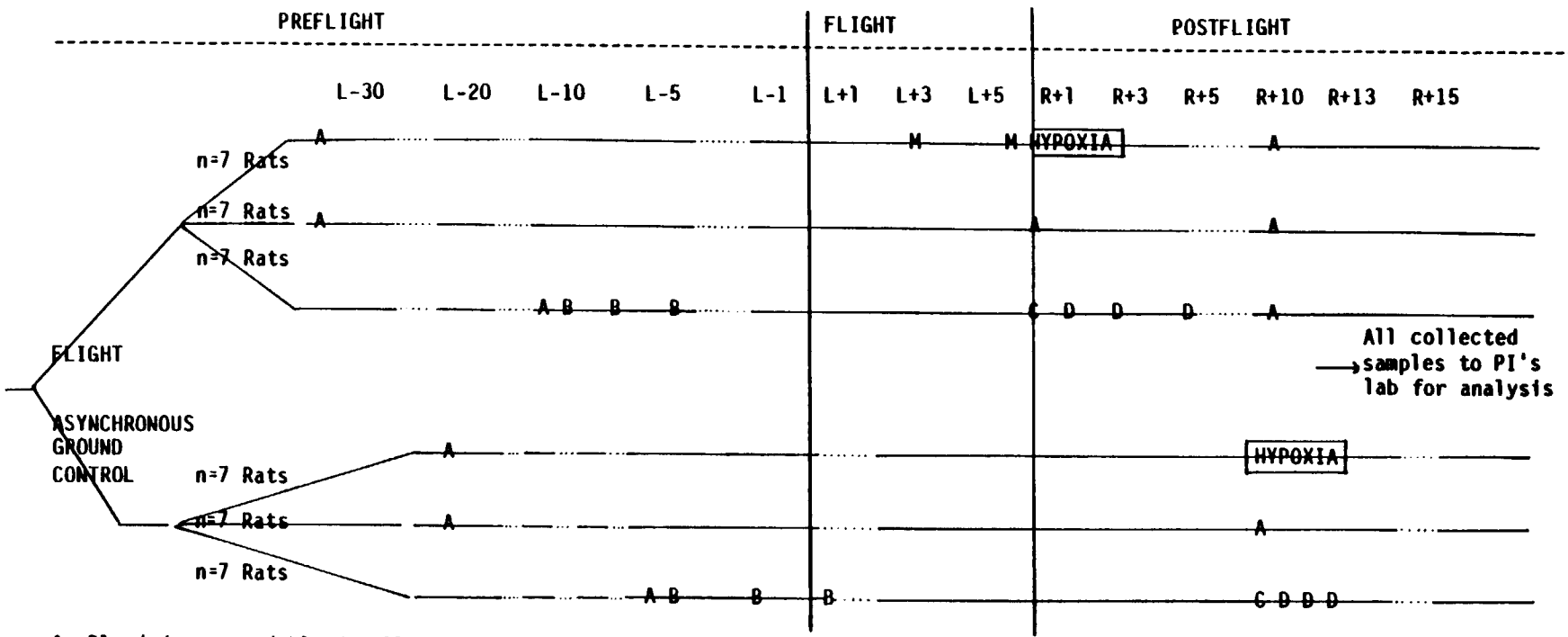
Definition phase activity resulted in the recommendation by the investigator that the inflight hypoxic chamber portion of the experiment be deleted. the current request by the investigator is that this hematologic challenge would be postflight only. During definition the concerns over the erythropoietin assay were eliminated.

EQUIPMENT: The PI has no flight hardware to be developed. The only experiment unique equipment to be developed is a biology kit. The required LSLE for this experiment includes the RAHF; GPWS; and the SMMI.

SUMMARY: This proposal addresses one of the fundamental observations of the response to weightlessness, decreased red blood cell mass. Recent advances have resolved previous concerns including the adequacy of the erythropoietin assay. The methods and techniques proposed in this investigation are excellent, and the results should test the rat as a model of human hematological changes in spaceflight. This research team would be combined with the other recommended rodent hematology team for a combined effort for flight. It is recommended that this experiment tentatively be selected for

flight on the understanding that it be combined with Dunn (781012), that it share animals aloft, that the inflight hypoxic stimulus be deleted, and that somewhat fewer flight animals be used.

EXPERIMENT FLOW DIAGRAM - ES 141



A: Blood drawn; red blood cell mass, plasma volume, hematocrit, reticulocyte index, reticulocyte classification, and red blood cell morphology determinations made postflight on collected samples.

B: Blood drawn for ^{51}Cr survival time; sample used for postflight assay.

C: ^{59}Fe injected.

D: Blood drawn; reticulocyte index, reticulocyte classification, red blood cell morphology, ^{59}Fe-RBC incorporation, ^{51}Cr survival time, haptoglobin, transferrin, ferritin, hemoglobin, and total protein determinations done postflight.

M: Body mass determinations performed.

Hypoxia: Animals are housed in hypoxic environment for 48 hrs.

PROPOSAL NUMBER: 781012

TITLE: Regulation of Erythropoiesis in Mice During Spaceflight, II

SPECIMEN: Mouse

PRINCIPAL INVESTIGATOR: Dunn, C.D.R., Ph.D.
AFFILIATION: Northrop Services Incorporated, Houston, TX

CoIs/AFFILIATIONS:
R. D. Lange, M.D./University of Tennessee Memorial Research Center, Knoxville, TN

BACKGROUND: Previous spaceflight crews have consistently exhibited a decrease in red blood cell (RBC) mass. The mechanisms of the loss are unclear. A decrease in erythropoiesis (red blood cell production) may play a role.

Factors affecting erythropoiesis include the nutritional status of the animal, plasma volume, hematocrit, O_2 availability, erythropoietin levels and cell-sensitivity to erythropoietin. Many of these parameters have been measured in spaceflight crews. More extensive data are needed. A controlled population such as mice could clarify the role of each parameter in the red blood cell mass decrease of spaceflight.

This experiment measures food and water intake, plasma volume and total blood volume. In addition, it measures parameters associated with red blood cell production such as erythropoietin levels, cell sensitivity to erythropoietin, and the functional state of circulating red blood cells.

PI OBJECTIVES: To measure changes in rate of red blood cell production in response to spaceflight. To assess the role of nutritional status (energy balance) and of hemo- concentration in the mouse's erythropoietic response to spaceflight.

PI HYPOTHESES: Animal studies and computer modeling data suggest that the mouse will undergo erythropoietic changes similar to man in response to spaceflight, and can thus be utilized to study this response. The suppression of red blood cell production following spaceflight may be related to a negative energy balance and/or changes in body fluids. RBC production during spaceflight may be decreased.

EXPERIMENT PLAN:

Preflight

Approximately 80 mice will be weighed daily. Food and water consumption will be measured. At L-8 days, 5 mice will be injected with ^{59}Fe to follow RBC production. At L-7 days, the 5 mice injected the previous day will be injected with ^{51}Cr-labelled RBCs for blood volume measurements. Each of these mice will then be bled to death, the blood sample quickly processed for hematocrit, reticulocyte index, hemoglobin p50, blood volume, and red cell production. The blood remaining will be pooled for the 5 mice, allowed to clot, centrifuged, and the serum stored for erythropoietin assay later. Cell cultures for erythropoietin sensitivity will be done.

Inflight

On days 1-2, 3-4, and 6-7 the preflight protocol (injections, blood collection and processing) will be repeated for 3 additional groups of 5 mice. Body mass determinations will be made on the mice prior to the blood collection. Cell cultures will not be performed inflight.

Postflight

The preflight protocol will be repeated: days 0-1, 1-2, 5-6, 14-15. Bone marrow and spleen cells will be cultured for erythropoietin sensitivity. Body weights, food and water consumption will be monitored daily.

Ground Control

Using the same protocol as described above, a ground control experiment is performed at KSC in near-synchronous time (24 or 48 hour delay). Research Animal Holding Facility (RAHF)-like cages and environment are requested.

Measurements

Type	Units
Body mass**	g
Food consumption**	g/day
Water consumption**	ml/day
Hematocrit**	%
Reticulocyte index**	%
Hemoglobin p50**	mmHg
Erythropoietin**	mU/ml
Blood volume**	ml
Red blood cell production**	%
Ambient temperature**	°C
Relative humidity**	%
Radiation levels in RAHF*	mR/h
Day/night cycle**	h
Erythropoietin sensitive cell cultures	colonies

* Data taken during flight procedures only.
** Data taken during flight and ground procedures.
All other data taken on the ground.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The Experiment Requirements Document incorporates a number of techniques for the measurement of erythropoiesis that were not a part of the original proposal. These include Hemoglobin p50, reticulocyte index (using a modification of the method developed for Spacelab human experiments), use of ^{59}Fe and ^{51}Cr-labelled erythrocytes simultaneously to measure erythrocyte production and blood volume. These methods were selected from the supporting studies performed by the investigators. The purpose of these studies was to find methods to minimize the number of animals required, to allow storage of samples for the time periods and temperatures required by the mission, and to minimize use of crew time.

SCIENCE: The basic hypothesis is good: that the red cell mass decrease observed to be related to spaceflight is due to decreased production of red blood cells, and the basic parameters affecting and controlling erythropoiesis are similar to those demonstrated experimentally in normal gravity (e.g., fluid loss, poor nutritional status, cellular insensitivity to erythropoietin). The scientific approach is very good. The protocol is designed to measure erythropoietic parameters preflight for baseline data, inflight when changes occur, and postflight during readaptation. The proposed experiment investigates changes shown to occur as a result of spaceflight and thus meets AO criteria 1 and 2. The experiment is straightforward, requiring only standard lab techniques, supplies and skills. The investigator has an excellent professional reputation. The experiment is in his primary area of expertise, and he has published numerous papers relevant to it. It therefore meets the payload objectives. It is responsive to the program objectives as well.

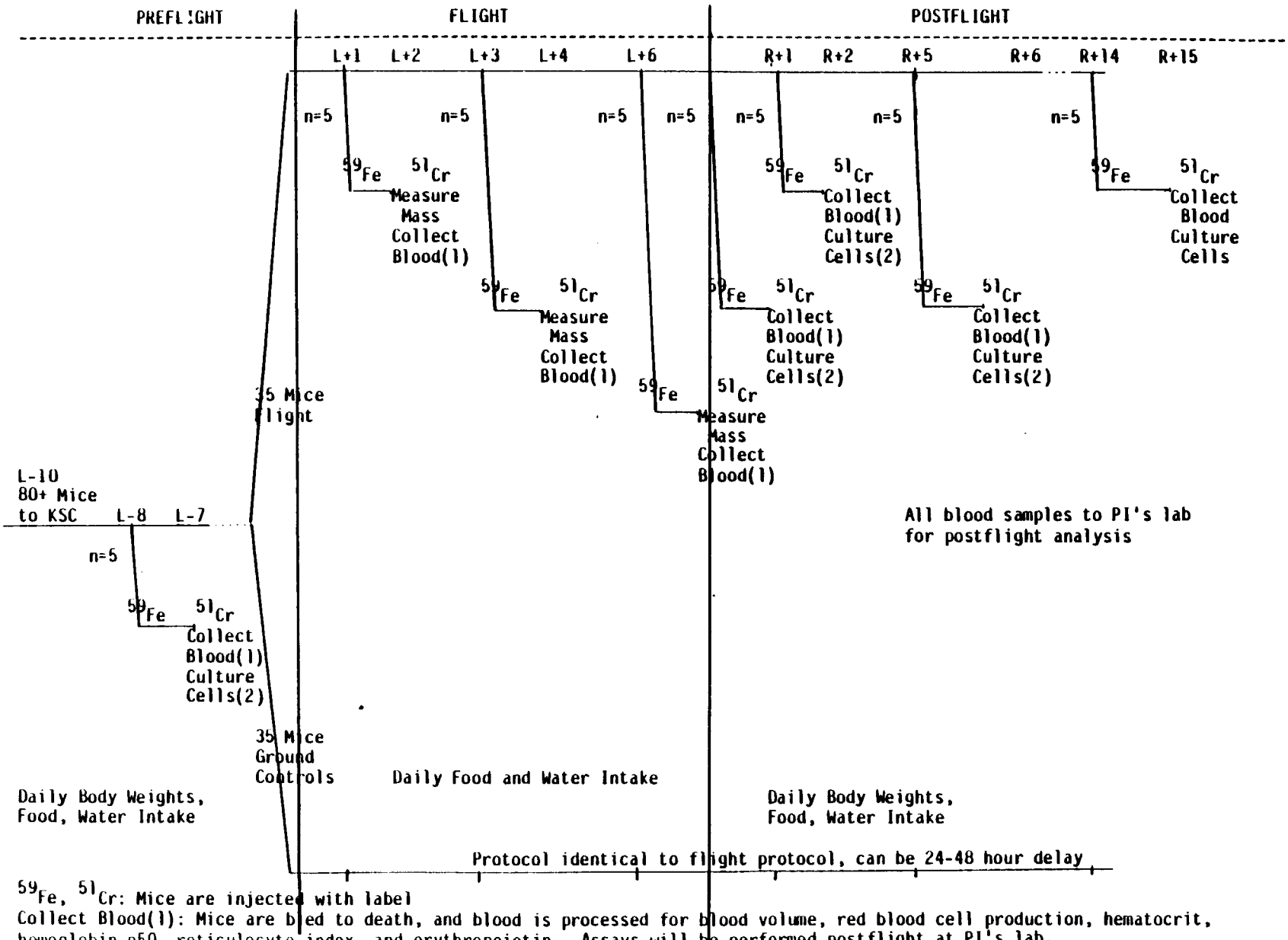
The original classification of this experiment was in category 3. This was due to the fact that this PI proposed 6 experiments, one of which used a technique that required further development. Development of that silicon membrane technique is required only for experiment 781014, and is not of concern to this study. During the definition phase activity, Dunn perfected his erythropoietin assay technique, removing any concerns about that portion of the experiment. Although originally classified as category 3, this experiment is now in an advanced state of readiness for flight.

As proposed, the experiment would use the laboratory mouse. To maximize the scientific yield of the hematology studies and to reduce duplication, NASA has proposed that this experiment and the experiment of Johnson (781141) be combined and that the combined experiment be performed on the laboratory rat. Preliminary talks with both investigators has confirmed the feasibility of this approach.

EQUIPMENT: The PI does not require flight hardware to be developed. The only experiment-unique equipment to be used is a hematology kit and a rodent restraint. Maximum use is made of LSLE: RAHF, GPWS, SMMI, hematocrit and laboratory centrifuge, refrigerator, freezer, radiation dosimeter and bioradiation storage.

SUMMARY: This experiment is a strong, careful, well-designed analysis of the effects of 0-g on the hematopoietic system. A marginal erythropoietin assay was responsible for the original categorization; this was much improved during definition. The silicon membrane technique will not be used in this experiment. The investigation can be carried out on rats rather than mice and still have a high likelihood of success. It is recommended that this experiment tentatively be selected for flight on the understanding that it be combined with Johnson (781141), employ rats, and consume somewhat less crew time.

EXPERIMENT FLOW DIAGRAM - ES 012



^{59}Fe, ^{51}Cr: Mice are injected with label

Collect Blood(1): Mice are bled to death, and blood is processed for blood volume, red blood cell production, hematocrit, hemoglobin p50, reticulocyte index, and erythropoietin. Assays will be performed postflight at PI's lab.

Culture Cells(2): Bone marrow and spleen cells are cultured for erythropoietin responsiveness.

MUSCLE

PROPOSAL NUMBER: 781120

TITLE: Protein Metabolism During Spaceflight

SPECIMEN: Human

PRINCIPAL INVESTIGATOR: Stein, T. D., Ph.D.
AFFILIATION: University of Pennsylvania, Philadelphia, PA

CoIs/AFFILIATIONS:
None

BACKGROUND: Dr. Stein plans to measure whole body protein synthesis, plasma albumin, plasma fibrinogen, immunoglobulin-g and hemoglobin synthesis rates by using a non-radioactive, isotope labeled amino acid, ^{15}N-glycine. The general approach employed requires only that the subject ingest by mouth 1.0 gram ^{15}N glycine in a capsule. Normal food intake (spacelab diet) is consumed in 1/12 portions each hour for 12 hours. All urine volume is collected and sample aliquots frozen for postflight analyses.

The scientific yield from this experiment will be an examination of the changes in protein balance during short duration spaceflight. Amino nitrogen not excreted is assumed to be incorporated into protein. Anticipated results relate to Skylab observations where a negative nitrogen balance of approximately 4 grams/day was observed. The nitrogen loss was generally reflected in the urine rather than in the fecal mass. This study is relevant to the Announcement of Opportunity (AO) criteria to expand current knowledge of prior observations from humans who have been exposed to weightlessness.

The specific contribution of this experiment is the whole body overview of changes in protein metabolism. It is simple to perform, non-invasive, and likely to provide significant new information.

PI OBJECTIVES: To measure the effect of spaceflight on human protein metabolism. To study the effect of spaceflight on plasma protein and hemoglobin synthesis. To determine whether spaceflight alters hemoglobin synthesis.

PI HYPOTHESES: Nitrogen retention is decreased during spaceflight. To investigate why this is so it is necessary to decide whether the decreased nitrogen retention is due to a relative decrease in protein synthesis or an increase in protein breakdown. Plasma protein synthesis rates are known to be altered by nutritional status and stress. Are similar changes found during spaceflight and can these changes be interpreted by comparison with the results of ground-based experiments? It may also be that the changes found with red cells during flight will correlate with alterations in red cell hemoglobin synthesis.

EXPERIMENT PLAN:

Preflight

Preflight studies shall begin 10-20 days prior to launch. The six member crew for flight are required preflight subjects. Each subject will follow an identical experiment routine.

One half hour after waking, a 10.0±3 ml blood sample shall be collected, centrifuged and the plasma frozen. The subject will then take by mouth a 1.0g ^{15}N-glycine capsule. He then voids and the urine void is frozen. For the next 12 hours, all excreted urine is collected and frozen. The subjects normal food intake for the 12 hour study is divided into 12 equal aliquots. One portion is taken/hour.

Twelve hours after the glycine ingestion, a 10.0±3 ml blood sample is collected, centrifuged and frozen. A final urine sample is collected and frozen.

Inflight

Inflight studies shall begin L+2. The six crew members shall follow the preflight routine. A crew member, rather than the PI staff, shall collect blood.

Postflight

Postflight studies shall begin R+7. The routine shall be identical to the preflight study.

Ground Studies

None planned.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The original proposed objectives have been maintained. Dr. Stein has simplified his original procedure and no longer proposes to give benzoic acid along with the glycine in hourly aliquots. One dose of glycine will be administered at the start of the experiment. Crew urine and blood sample requirements are reduced. Total body water determination has also been omitted. Early in definition there was some concern that the proposed technique lacked adequate resolution. However, recent results from PI's lab clearly establish that he has adequate resolution.

SCIENCE: Dr. Stein's investigation will yield significant new information concerning nitrogen loss, a phenomenon observed in earlier manned spaceflight missions, and whole-body protein turnover. Measurements that will be made include liver protein synthesis, catabolism, and amino acid pools, whole-body nitrogen pools, muscle protein catabolism, and collagen protein catabolism. The data generated from this experiment directly relate to other areas of study, particularly changes in lean body mass and body weight, muscle atrophy, collagen breakdown, whole-body energy requirements, and nutritional requirements. The nitrogen losses, observed in previous space-flight crew members, have been attributed to hypothesized and observed changes in muscle mass. This category 1 experiment would begin to clarify the extent to which the nitrogen losses can be related to muscle atrophy and to changes in liver protein metabolism. It is consistent with the first announcement of opportunity evaluation criterion, and the payload and program objectives. Furthermore, it is the most crew time effective, non-invasive analysis of whole-body protein turnover relative to other studies which were proposed for LS-1.

EQUIPMENT: This investigation requires only NASA supplied LSLE items. No PI unique hardware is needed. The LSLE items are the rack mounted centrifuge, freezer, the inflight blood collection system, the hematocrit centrifuge, and the urine monitoring system.

SUMMARY: This proposal is a non-invasive analysis of body protein turnover to determine if the nitrogen loss seen in Skylab is due to reduced nitrogen uptake or increased protein destruction. It makes efficient use of crew time and has a high probability of success. It is recommended that this experiment tentatively be accepted for flight on the understanding that he share specimens aloft and delete the ground-based study dealing with "jet lag".

FLOW CHART AND MEASUREMENT TABLE FOR EXPERIMENT #120 - STEIN

TESTS	Preflight	Inflight	Postflight	
		Launch	Recovery	
	L -15	L +2	L +9*	R +7
URINE COLLECTION AND PRESERVATION**				
Pool and freeze urine voids	6	6	6	6
GLYCINE INGESTION				
1.0g, 99 percent ^{15}N-glycine	6	6	6	6
BLOOD COLLECTION & STORAGE**				
10 ml samples Centrifuge Measure hematocrit	6	6	6	6
FOOD INGESTION				
12 equal aliquots in 12 hours Record total dietary intake (gN, Kcal/day)	6	6	6	6

* Repetition on a 10-day flight

** Plasma and urine samples will be analyzed to determine synthesis rates of whole-body protein, albumin, fibrinogen, immunoglobulins and hemoglobin, and breakdown rates of muscle protein and collagen

PROPOSAL NUMBER: 781247

TITLE: Skeletal Myosin Isoenzymes in Rats Exposed to Zero-Gravity

SPECIMEN: Rat

PRINCIPAL INVESTIGATOR: Hoh, J. F. Y., Ph.D.
AFFILIATION: University of Sydney, Sydney, Australia

CoIs/AFFILIATIONS:
None

BACKGROUND: Skeletal muscle fibers exist in two forms, "slow-twitch" and "fast-twitch". The two forms develop similar contractile forces but with different speeds. The speed of contraction is related to the subunits of myosin, one of the main muscle fiber proteins found in any given fiber. The myosin subunits or isoenzymes exist in five separate forms, which differ in structure and in enzyme activity.

The synthesis of myosin isoenzymes is regulated by the nervous system via the pattern of impulse activity of the motor neurons. Slow-twitch muscle fibers are stimulated at a low tonic discharge frequency. Fast-twitch fibers are stimulated at a high phasic discharge frequency.

Slow-twitch fibers support the body against gravity, while fast-twitch fibers are related to movement of the body. Experiments where the neural inputs are modified by nerve cross-union, spinal cord transection, or imposed patterns of electrical stimulation have demonstrated changes in isoenzyme patterns. Nerve cross-union experiments between fast and slow-twitch fibers lead to reversal of contractile properties of the fibers, accompanied by the appropriate changes in electrophoretic properties of the isoenzymes.

In 0-g, neural stimulation of slow-twitch fibers should be minimal. It is expected that the isoenzyme pattern will be altered accordingly, that is, decreases in slow-twitch isoenzymes will be seen. Preliminary data from rats from Cosmos 1129 by Chiu et al. support this point of view.

PI OBJECTIVES: To determine whether lack of gravity will modify muscle myosin isoenzymes. To determine whether slow-twitch antigravity muscles will tend toward fast-twitch with prolonged exposure to 0-g.

PI HYPOTHESIS: Reduced usage of anti-gravity muscles will result in muscle alterations toward fast-twitch muscle type.

EXPERIMENT PLAN:

Preflight

Ten rats, 4-5 week old females, will be used. Animals will be weighed for assurance that all are approximately equal and stable in weight.

Inflight

Five animals will be flown and housed in the Research Animal Holding Facility (RAHF). Activity levels for the group will be monitored for 5 minutes daily by crew member. Data from the RAHF's activity monitors will be collected. A one hour videotape or 16 mm film to record the animals' motor activity is requested at the beginning of flight and again near the end.

Postflight

Animals will be removed from the shuttle as soon after flight as is possible and returned to Hangar L for postflight experimentation. Body weights will be determined. Flight animals will be anesthetized. The muscles will be dissected from both hind legs of each animal. Muscles from one leg of each animal will be incubated for a short period in ^{3}H-amino acids, then packed in iced glycerol. Muscles from the other leg of each animal will immediately be packed in the iced glycerol. All samples will be returned to the PI's lab for myosin isoenzyme profiles and ^{3}H-incorporation analyses. Ground control animals will be treated identically.

Ground Controls

The remaining five animals will be used for the identical ground control experiment, performed in near-synchronous time (24 to 48 hour delays) at KSC.

Measurements

Type	Units
Myosin isoenzymes profile	N/A
Animal activity*	counts
Body weight	g
Incorporation of ^{3}H-amino acids into isoenzymes	CPM
Muscle weight (soleus)	mg
Muscle weight (extensor digitorum longus)	mg
Movie film activity record*	minutes
Voice tape activity record*	minutes
Food consumption*	g/day
Water consumption*	ml/day
Temperature**	°C
Relative humidity**	%
Photoperiod**	h

* Data taken during flight procedures only.
** Data taken during flight and ground procedures.
All other data taken on the ground.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The Experiment Requirements Document (ERD) reflects two major changes from the original proposal. The requirement for a 1-g animal centrifuge was deleted. The postflight procedure has been improved. Postflight, the soleus muscle from one leg of each animal is incubated in

tritiated amino acids to measure their incorporation into newly-forming myosin isoenzymes. The muscles from both legs are then prepared for gel electrophoresis of the isoenzymes as described in the proposal. Incorporation of the tritiated amino acids into individual isoenzyme gel bands will be used to assess the changes in isoenzyme profile.

SCIENCE: This experiment proposes to use the rat to study zero-g caused changes in the structure of weight-bearing muscles, including the relative distribution of muscle fibers between the slow and fast-twitch type. The relative distribution of these fibers and the enzymes associated with them will provide data about the adaptation of muscle to the decreased load of weightlessness. The experiment has a strong, well received hypothesis, supported by ground-based experiments, and, to some extent, by previous spaceflight (Cosmos) data. This is one of the most exciting of the muscle experiments and should provide a good data base for continued studies on the adaptation of a gravity sensitive system to weightlessness.

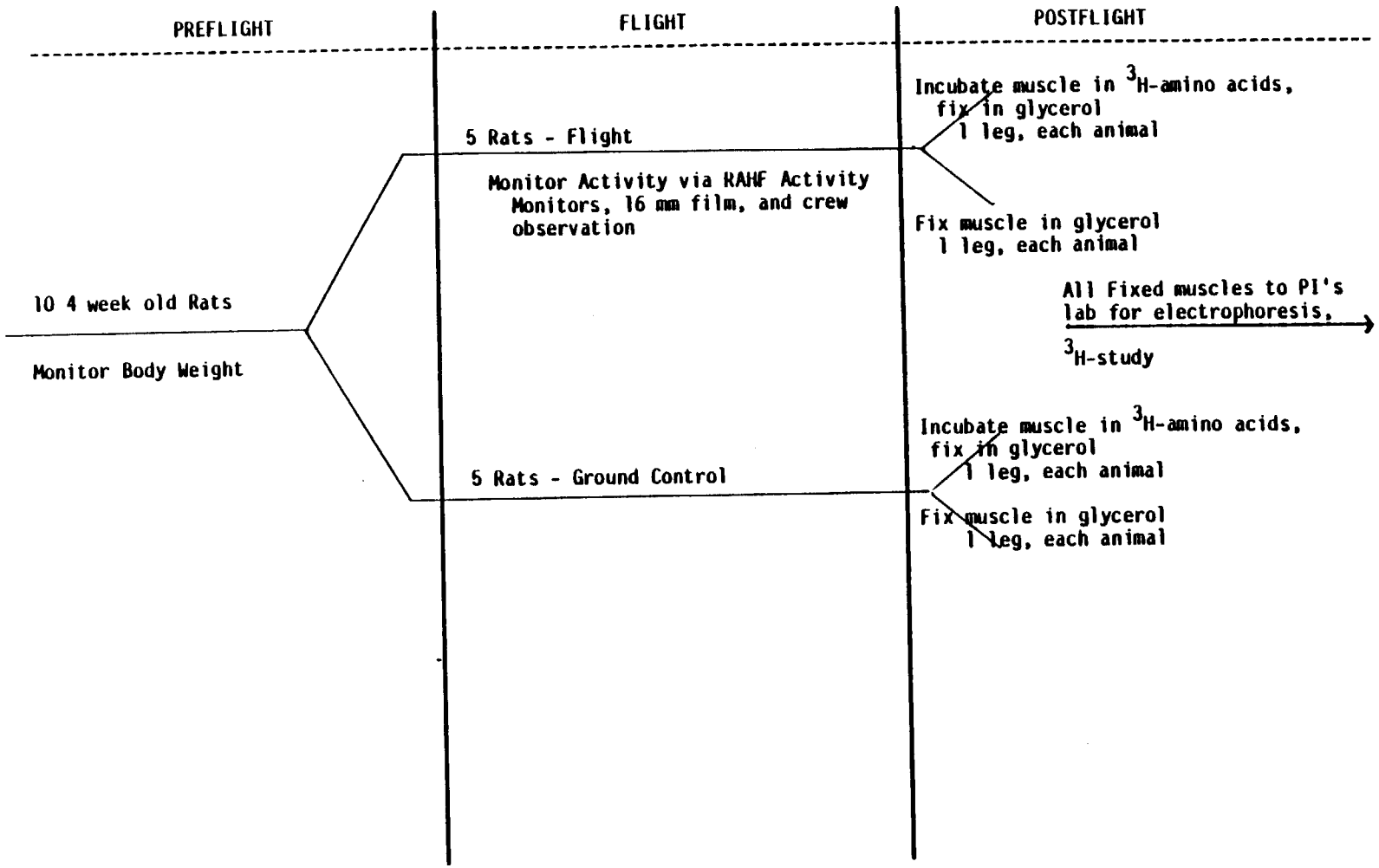
This proposal is responsive to the objectives of the program and the results might be applicable to some questions about muscular adaptation to weightlessness in man. This experiment has a very good chance of providing significant data, and has a high likelihood of success. The experiment can be conducted almost completely as proposed, and can share animals with other investigations.

The results of the definition phase activity have confirmed the original contention that this experiment is in a high state of readiness for flight.

EQUIPMENT: The PI has no hardware to be developed. The experiment unique equipment includes: 16-mm film cassettes. The LSLE required is: RAHF; voice recorder; 16-mm camera with mount and mirror assembly; and the DEMS.

SUMMARY: The relative distribution of slow and fast-twitch fibers and their associated isoenzyme patterns will provide data on muscle adaptation in weightlessness. This highly rated experiment has a good hypothesis which is supported by both ground-based experiments and previous spaceflight data. Resource requirements are reasonable and the PI has an excellent record in this research area. It is recommended that this investigation tentatively be selected for flight on the understanding that it share animals, eliminate the need for a 1-G centrifuge, and upgrade some postflight techniques.

EXPERIMENT FLOW DIAGRAM - ES 247



PROPOSAL NUMBER: 781127

TITLE: Effect of Zero-Gravity Exposure on Biochemical and Metabolic Properties of Skeletal Muscle

SPECIMEN: Rat

PRINCIPAL INVESTIGATOR: Baldwin, K. M., Ph.D.
AFFILIATION: University of California, Irvine, CA

CoIs/AFFILIATIONS: None

BACKGROUND: It has been proposed that a loss of muscle mass in astronauts (Skylab missions 2, 3 and 4) during weightlessness produces the observed loss of strength and endurance, particularly in the antigravity muscles. One explanation is that exposure to 0-g results in the removal of sufficient stress or tension on the muscles to maintain adequate levels of protein or enzyme activity of certain subcellular systems. That is, most cellular proteins have a short half-life (5-7 days) so that overall reductions or loss of the enzyme activity in muscle in weightlessness should occur whenever there are insufficient substrate concentrations or renewals of proteins. This leads to reductions in contractual output (endurance) of the muscle. It is expected that weightlessness causes a reduced capacity for oxidative metabolism in skeletal muscle leading to a greater dependence on the anaerobic energy expenditure of glycogen which ultimately limits the endurance capacity of the individual.

PI OBJECTIVES: To determine impairments in the functional capacity of skeletal muscle due to 0-g exposure utilizing a rat model. To determine the treadmill running endurance capacity of rats following exposure to space-flight conditions. To determine alterations in tissue glycogen depletion in rested vs. exercised rats following exposure to 0-g. To measure the oxidative capacity of muscle homogenates with ^{14}C-pyruvate and ^{14}C-palmitate as substrate. To assess alterations in mitochondrial and glycogenolytic enzymes due to 0-g by histochemical and biochemical methods.

PI HYPOTHESES: 0-g exposure will result in a decrease of mitochondrial enzyme levels, primarily in the oxidative fiber-type portions of antigravity muscles in the rat. The mitochondrial reductions will lower the muscles' capacity for free fatty acid (FFA) oxidation, in turn causing a loss of stamina during activity. This decreased endurance capacity will be due to the increased utilization of carbohydrate stores as the primary source of energy, thereby causing a rapid depletion of such stores.

EXPERIMENT PLAN:

Preflight

Four weeks prior to launch 50 female rats (Wistar, 200g) will be obtained and screened for running ability on a rodent treadmill. Thirty-six animals will be selected as willing runners and randomly assigned to one of two groups, Normal Control (NC; N=18), and 0-g Exposed (ZG; N=18).

Inflight

No inflight procedures are required for the 18 ZG animals other than normal Research Animal Holding Facility (RAHF) maintenance. The control animals (NC=18) will be maintained at KSC in conditions similar to those of the ZG group for the duration of the flight.

Postflight

Postflight recovery of the specimens shall occur 3-5 hrs. after reentry and testing shall commence immediately. The two groups, ZG and NC, will be further divided into three subgroups: Endurance capacity, Rested, and Exercise for 30 minutes.

In subgroup (A), six ZG and six NC rats will be subjected to a standardized treadmill test (TBD) which is identical to the preflight test and then sacrificed by decapitation. The muscle tissues will be dissected, quick frozen, and stored at -70°C for subsequent muscle enzymology and histochemistry at the investigator's laboratory.

In subgroup (B) six ZG and six NC rats will be anesthetized (Nembutal) while at rest, and muscle tissues will be removed for analyses. In subgroup (C), six ZG and six NC rats will be subjected to 30 minutes of treadmill running after which they will be anesthetized (Nembutal) and their tissues removed for biochemical analyses.

Tissue preparations and minimal biochemical manipulations will occur at KSC during days R + 1 and R + 2. The remaining analyses will be performed at the investigator's laboratory.

Ground Controls

The 18 Normal Control (NC) animals maintained under RAHF-like conditions at the KSC Life Sciences Facility will provide the control values. The mature female Wistar rat maintains a reliably stable caloric intake, therefore, pair-feeding of the NC and ZG animals will not be required. Launch and reentry simulations are requested for the NC group to control for stress effects. The NC and ZG animals will be processed simultaneously postflight.

Measurements

Type	Units
Preflight endurance (running time)	min
Postflight endurance (running time)	min
Oxidative capacity of muscle homogenate	uMol/g/min
Muscle glycogen	uMol/g tissue
Muscle protein	mg/g
Liver glycogen	uMol/g tissue
Blood glucose	uMol/ml
Blood lactate	uMol/ml
Plasma free fatty acids (FFA)	uMol/ml
Muscle histochemistry	N/A
Muscle hexokinase	uMol/g/min
Muscle citrate synthase	uMol/g/min
Gastrocnemius weight	mg

Type	Units
Soleus weight	mg
Preflight animal weight	g
Postflight animal weight	g

* Data taken during flight procedures only.
** Data taken during flight and ground procedures. All other data taken on the ground.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: Although the basic hypotheses and objectives of this proposal have remained the same through definition, the study protocol has been reduced. Originally, female Wistar rats were divided into five equal groups (n=16 each): (1) 0-g sedentary, (2) 0-g trained, (3) vivarium-control sedentary, (4) vivarium-control trained, and (5) flight synchrony control. Groups (2) and (4) were subjected to a 12-week treadmill running training for conditioning. This has been changed to a total of 36 female rats divided into two groups: a normal control group (n=18) and a 0-g exposed group (n=18) subjected to 15 minutes of exercise 3 times weekly for 3 weeks, followed by endurance tests. Rats are only maintained (no tests) during flight.

According to the ERD, the two groups are divided into 3 subgroups (n=6 each) postflight: endurance capacity, rested, and 30-min. exercised. Originally, five rats from each group were sacrificed immediately following a duration treadmill run. The remaining 6 animals were sacrificed 21 days later. The ERD calls for the following: Group (A) to undergo treadmill testing identical to preflight exposure with hind limb muscles and liver samples to be obtained and stored at -70°C; Group (B) rats are sacrificed for muscles; Group (C) animals will be exercised, anesthetized, and sacrificed. The postflight biochemical/histochemical measurements have been changed according to the following list:

Originally proposed	Presently proposed
muscle actomyosin	
muscle glycogen	muscle glycogen
muscle protein	muscle protein
liver glycogen	--
blood glucose	--
blood lactate	--
muscle weights	muscle weights
plasma-free fatty acids	--
muscle histochemistry	muscle histochemistry
muscle hexokinase	muscle hexokinase
muscle citrate synthetase	--
muscle cytochrome C	
carnitine palmityl transferase	
muscle glycolytic and glycogenolytic capacity	
muscle malate dehydrogenase	
muscle RNA, DNA	
muscle hydroxyproline	
muscle water content	

SCIENCE: Baldwin's proposed experiment will yield significant information regarding the effects of weightlessness on the biochemical and metabolic properties of skeletal muscle. He will examine the effects in rested and exercised rats by assessing tissue glycogen depletion and alterations in mitochondrial and glycogenolytic enzymes by histochemical and biochemical methods.

The experimental design provides an adequate test of all hypotheses. An animal model for exercising astronauts will be verified. The investigator is experienced in muscle and exercise physiology and he is enthusiastic about the space program. His laboratory is active and is being funded by sources other than NASA.

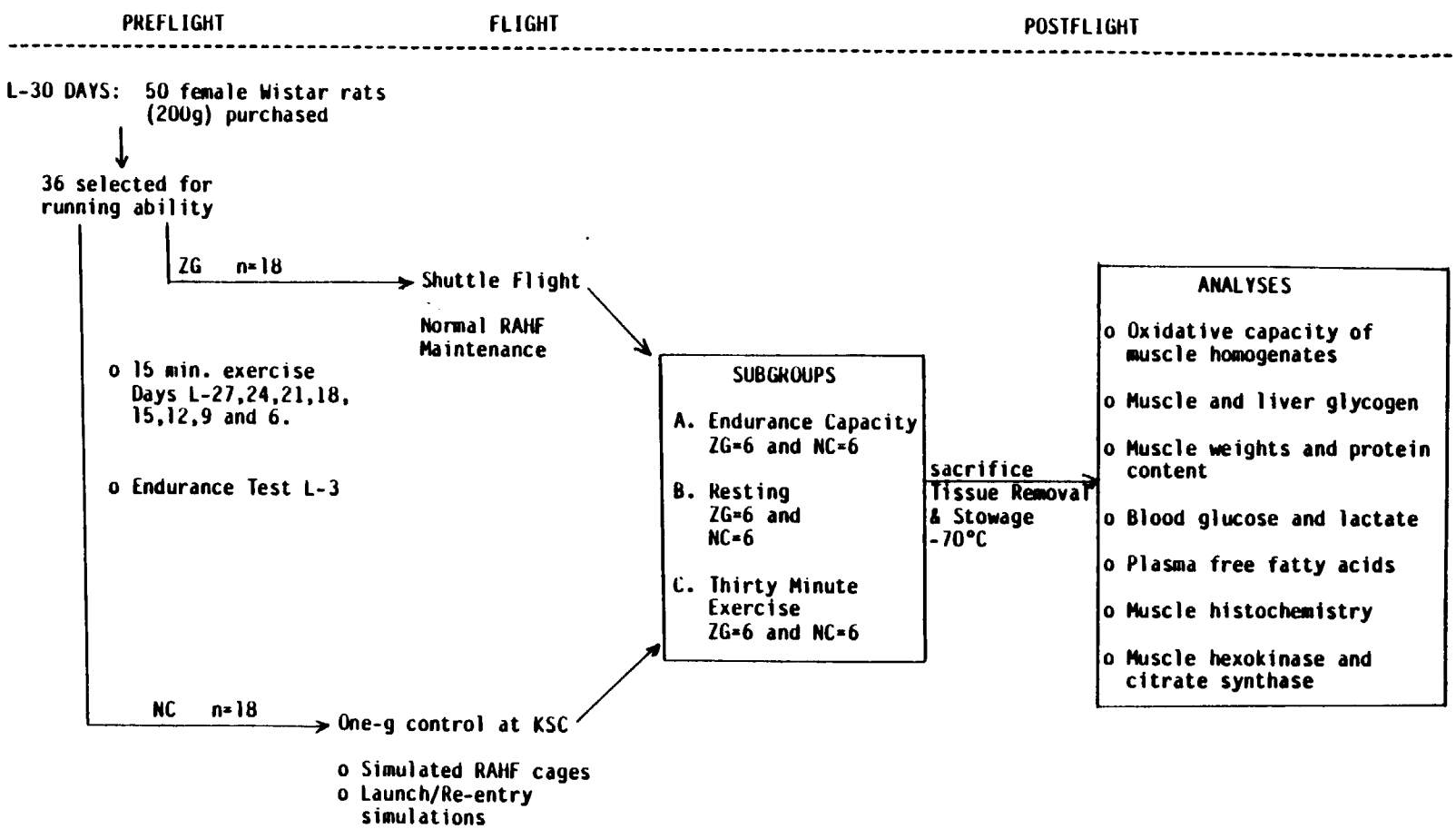
The experiment was originally assigned to category 3, but the problems with this experiment have been resolved during the definition phase of development. This experiment is responsive to AO evaluation criteria 1 and 2: physiological performance observed in humans who have flown in space, and investigation of a biological phenomenon known to occur as a result of the space environment. It meets the payload and program objectives.

Because of the limited space to keep animals on-board Spacelab and the need therefore to optimize resources, an animal sharing plan has been proposed by NASA. It is recommended that Baldwin share his rats with several other investigators.

EQUIPMENT: Dr. Baldwin does not require any hardware to be developed for this experiment.

SUMMARY: This investigation offers a broad biochemical assessment of skeletal muscle after exposure to weightlessness. More recent laboratory work has eliminated the earlier concerns regarding quantitative morphological studies which have now been strengthened. Exhaustive biochemical analyses are planned postflight and appropriate ground controls and supporting studies are also included. The methods proposed are state-of-the-art and are currently being used in the PI's lab. The experiment has a very high chance of resolving the hypotheses. It is recommended that Dr. Baldwin's experiment tentatively be selected for flight on the understanding that it share animals, use somewhat fewer flight animals, and combine with other experiments to eliminate redundant measurements.

EXPERIMENT FLOW DIAGRAM - ES 127



PROPOSAL NUMBER: 781303

TITLE: Electron Microscopy, Electromyography and Protease Activity in Rat Hind Limb Muscle

SPECIMEN: Rat

PRINCIPAL INVESTIGATOR: Ellis, S., Ph.D.
AFFILIATION: NASA-ARC, Moffett Field, CA

CoIs/AFFILIATIONS:
D. A. Riley, Ph.D./University of California, San Francisco, CA

BACKGROUND: Muscle changes occurring during Skylab missions are predictable based on terrestrial studies done by denervation. Denervation involves cutting the nerve going to the muscle. This causes immediate cessation of muscle contractile activity and degeneration sets in. Necrosis of the muscle results with fat and connective tissue replacing muscle mass. The rate and degree of muscle fiber destruction is greatest for denervation. The changes in muscle which might be prompted by hypogravity are:

1. Myofibril dissolution
2. Mitochondrial degeneration
3. Increased production of collagen
4. Increased lysosomes and autophagic vacuoles
5. Hypertrophy of Golgi apparatus
6. Invasion of macrophages
7. Overall loss of muscle mass and myelinated nerve fiber

From Cosmos 782 and 936 data it is known that rat soleus muscle is susceptible to hypogravity atrophy, with 32% reduction in weight and a 22% reduction in cross-sectional area. This was accompanied by a significant increase in the activity of mitochondrial and nicotinamide adenine dinucleotide (NAD) bound glucophosphate dehydrogenase. A decrease in Krebs cycle enzymes was also found. These signs were thought to be a reflection of hypodynamia, similar to restricted movement. Disuse atrophy can display significant elevations in the concentration of muscle proteases which play a critical role in the degradation of muscle tissue.

PI OBJECTIVES: To define the nature and progression of muscle atrophy in hypogravity, in morphological and histochemical, proteolytic and myoelectric terms and for the soleus nerve in morphological and histochemical terms. To assess in the above terms, the trauma of reentry to debilitated skeletal muscles 2 days postflight. To assess the degree of recovery 26 and 45 days postflight.

PI HYPOTHESES: Prolonged exposure to hypogravity causes atrophy of the antigravity muscles (soleus and gastrocnemius). The tonically active slow soleus will deteriorate more rapidly than the physically active fast gastrocnemius. The antigravity muscles should undergo morphological changes due to four circumstances: launch stress, inflight atrophy, reentry stress injury and lastly postflight repair. The amount of soleus atrophy will be proportional to the degree of muscle inactivity as monitored by electromyography. As a consequence of weightlessness the spectrum of proteases in the subcellular organelles of these muscles should increase several fold.

EXPERIMENT PLAN:

Preflight

Twenty-nine female rats will be habituated in Research Animal Holding Facility (RAHF)-like cages prior to flight. Of this number, five will be implanted with gross electromyographic (EMG) transmitters. The activity patterns of these animals will be monitored during the habituation period.

Inflight

The twenty-nine animals will be divided into three groups. Group 1 consists of the five implanted rats. These will have their EMG signals recorded and a videotape made of the physical activity on a daily basis. Group 2 will consist of eight rats which will be maintained within the RAHF for the entire flight. Group 3 will consist of the remaining sixteen rats. Four each of these will be sacrificed on days L+1, 3, 5, and 7. Soleus and gastrocnemius muscles will be removed and returned for analysis.

Postflight

Thirteen rats and muscle samples are recovered from the Spacelab. The five implanted rats will be monitored for EMG and physical activity for forty-five days, at which time they will be sacrificed and the hind leg muscles removed. The other eight rats will be sacrificed, four each, at 2 days and 26 days postflight.

The muscles will be examined morphologically, histologically, and biochemically.

Ground Control

Three types of ground controls are proposed. The first type is a vivarium control to be performed at the time of the mission. The other two are a delayed Actual Flight Profile (AFP) control and another vivarium control. These last two will be performed at some time postflight. Each group has the same number of specimens as the flight group (n=29).

Measurements

Type	Units
EMG of hind limb muscle (soleus)**	Video record of movement**
Food consumption**	g/day
Water consumption**	ml/day
Body mass*	g
Activity from cage**	counts/day
Morphology of muscle and nerve fibers (light microscopy)	N/A
Histochemical analyses:	
Mitochondrial enzymes	N/A
Lysosomal enzymes	N/A
Glycogen	N/A

Type	Units
Biochemical analyses:	
Cathepsin B,D, DAP I,II,III,IV	uMol
Calcium activated proteases	uMol
LDH isozymes	uMol
Malate dehydrogenase	uMol
Electron microscopy:	
Myofibrils	
T&SR membranes	N/A
Mitochondria	
Macrophages	N/A
Lysosomes	
Collagen and	N/A
Autophagic vacuoles	
neuromuscular	N/A
Golgi apparatus junction	
Temperature**	°C
Relative Humidity**	%
pO_2**	mmHg
pCO_2**	mmHg
Light cycle**	h
Acceleration during EMG*	g
Acoustic noise during EMG*	dB
Radiation dose (mission)*	RAD

* Data taken during flight procedures only.
** Data taken during flight and ground procedures.
All other data taken on the ground.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: There are two differences between Ellis' proposal and the Experiment Requirements Document (ERD). The first is the addition of 4 rats to the flight group. These four extra animals were added so that the EMG-implanted rats could be allowed to survive until R+45 days rather than R+26 days. The four extra animals will be sacrificed on R+26 days in their place. This was done to allow better correlation with previous Cosmos data which used a similar postflight sacrifice schedule.

The second change added a delayed actual flight profile (AFP) control study and a delayed vivarium ground control study to eliminate stress effects imposed by launch and reentry.

This experiment should be easily combined with other rat experiments.

SCIENCE: The PI plans to excise rat antigravity muscles and study the changes induced by weightlessness by a variety of complementary analyses. Muscle samples taken during flight will be returned to ground-based laboratories for analysis. Periodic monitoring of the EMG activity of the soleus muscle will define patterns of muscle use. The results should establish whether muscles do atrophy in the weightless rat, and whether this is similar to or distinct from denervation or immobilization atrophy in humans.

This experiment is well planned with adequate numbers of specimens and a design which follows muscle changes both during flight and postflight to recovery. Background data from Cosmos supports the PI's hypotheses and the experimental protocol will accomplish the experiment objectives. The analytical methods are well worked out with the exception of the EM image analysis, which is part of a supporting study.

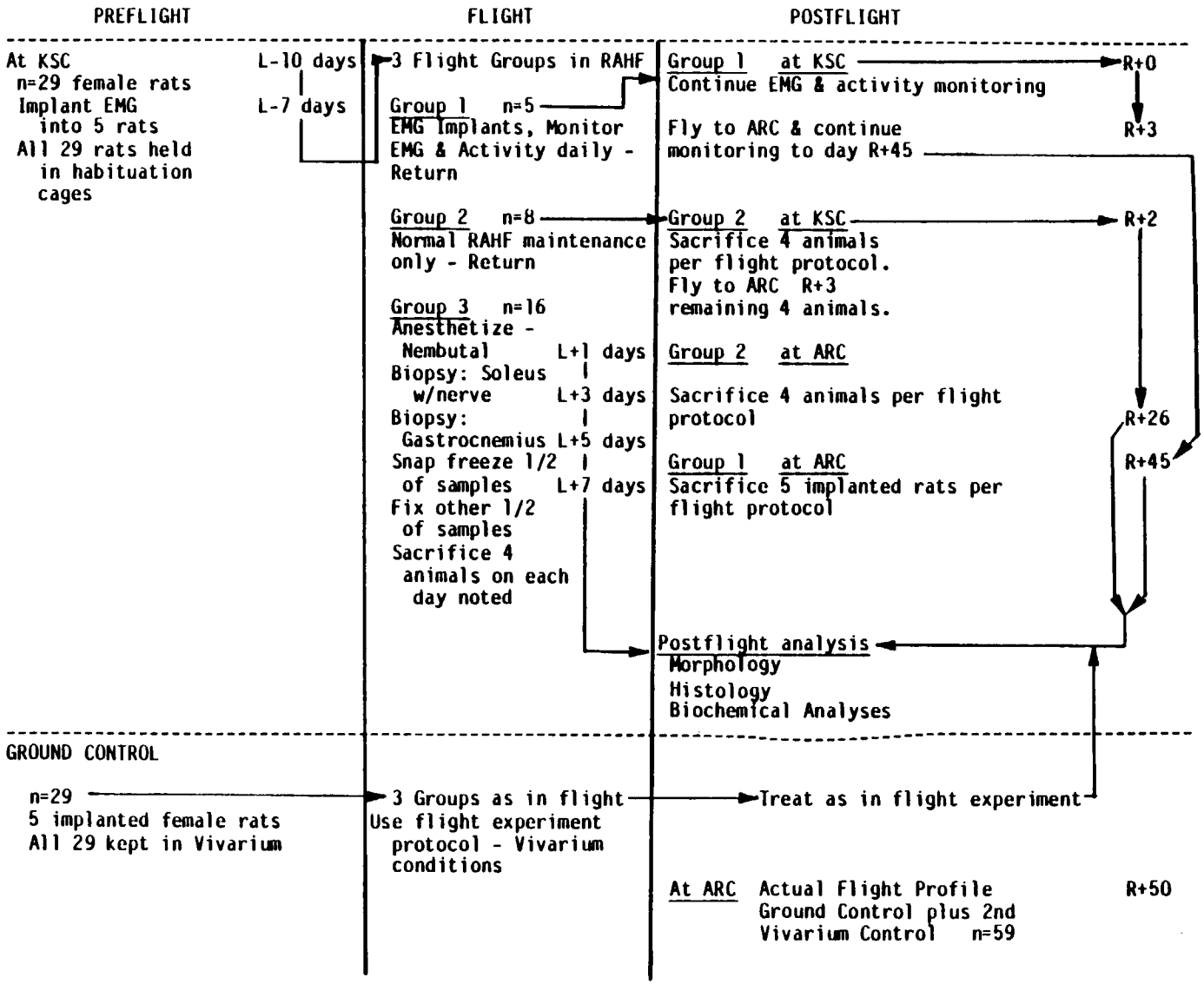
The PI is a well-respected investigator who has had prior spaceflight experience on Cosmos. He and his Co-I are completely capable of performing the analyses proposed and resolving the hypotheses. This experiment complements the other muscle experiments very nicely; the entire group of muscle experiments offers an integrated approach to the question of weightlessness atrophy and they should provide a set of benchmark data useful for examining countermeasures. Because Ellis plans to sacrifice rats in space and very few other PIs want to do that, his sample size had to be reduced. Further, he will be sharing the few animals he has with other investigators.

The phenomenon under investigation has been observed in humans as a result of spaceflight, so AO evaluation criterion 1 has been met. In addition, the investigation is consistent with the payload and program objectives.

EQUIPMENT: The PI requests that NASA design and develop the flight biotelemetry system for EMG. The BTS is already under development. Experiment-unique equipment for the uncompromised experiment was to have been a video camera, oxygen and carbon dioxide monitors, EMG transmitters, dissection and histology kits, and rodent restraint system. The LSLE needed was RAHF, GPWS, SMMI, DEMS, video camera mount, mirror assembly and recorder, dissecting microscope, snap freezer, refrigerator, -20°C freezers, mini-osciloscope, radiation dosimeter, and voice recorder.

SUMMARY: This experiment adds much to the existing complement of muscle experiments particularly in the areas of electron microscopy and histochemistry. Small changes to the protocol recommended during definition should enhance the scientific value of this experiment. These involve adding several animals to the flight group and arranging the protocol to be more compatible with the Cosmos findings. It is recommended that this experiment tentatively be selected for flight on the understanding that the investigation share animals aloft, combine with other experiments, use fewer animals overall, eliminate redundant measurements, and accept the possibility of eliminating the electromyographic data.

EXPERIMENT FLOW DIAGRAM - ES 303



PROPOSAL NUMBER: 781305

TITLE: Pathophysiology of Mineral Loss During Increased Urinary & Fecal Calcium Excretion

SPECIMEN: Human

PRINCIPAL INVESTIGATOR: Arnaud, C. D., M.D.
AFFILIATION: University of California, San Francisco, CA

CoIs/AFFILIATIONS:
C. E. Cann, Ph.D., University of California, San Francisco, CA
S. B. Arnaud, M.D., University of California, San Francisco, CA
E. M. Holton, Ph.D., NASA-ARC, Moffett Field, CA

BACKGROUND: Dr. Arnaud proposed a comprehensive study of calcium turnover and expected bone resorption. The approach is based on the immunoassay of parathyroid hormone and calcitonin, as well as the quantification of vitamin D metabolites. Calcium uptake would be evaluated using dual stable isotopes (^{46}Ca and ^{48}Ca). The proposal requires a controlled metabolic balance study. The proposal is responsive to the Announcement of Opportunity (AO) criteria to extend prior spaceflight findings concerning alterations in calcium balance.

PI OBJECTIVES: To determine the changes which occur and the time of onset of changes in calcium turnover, calcium absorption and bone resorption during spaceflight. To measure the changes in circulating levels of calciotropic hormones which occur during spaceflight. To use the correlated information from objectives 1 and 2 to develop a model of calcium and bone metabolism in spaceflight.

PI HYPOTHESES: Weightlessness causes an increase in bone resorption. This increase is expected to be primary, that is, the onset is within 1 to 2 days after exposure to null gravity. There are significant changes in serum ionized calcium and calciotropic hormones in response to the increase in bone resorption. These changes occur within hours of the changes in bone metabolism. Calcium turnover and intestinal absorption change during spaceflight in response to bone and endocrine changes.

EXPERIMENT PLAN:

Preflight

Six weeks preflight, four crew members will begin the flight stabilization diet. On L-18, 10, and 4, 20.0 ml fasting blood samples will be collected for measurement of parathyroid hormone, calcitonin, Vitamin D, serum Ca, Mg, and P.

Following the blood collection on L-18, each subject will be given a capsule containing ^{48}Ca-enriched $CaCO_3$. Then each subject will receive an injection of ^{46}Ca, a stable tracer. Approximately 4 hours after injection of ^{46}Ca, a 6.0 ml blood sample will be collected. On days L-14, 15, 16, 17, at the same time as the day L-18 blood draw, 6.0 ml blood will be collected from each subject. All blood samples will be centrifuged and serum/plasma removed for

storage. Endocrine and calcitonin samples will be stored at -70°C; tracer samples will be stored at -20°C.

Inflight

Procedures for inflight are the same as preflight. 20.0 ml samples for endocrine samples will be collected on Mission Days (MD) 3 and 6. On MD 4, a 60 ml fasting blood sample will be collected. Subjects will then take the $^{48}CaCO_3$ capsule. Between 1.5-2.0 hours after the oral tracer is given, a ^{46}Ca injection will be given to each crew member intravenously. Following tracer injection (t=0), 6.0 ml blood will be collected as follows: t=4.0±0.5 hrs., 10±1 hrs., 20±3 hrs., 44±3 hrs. and 68±3 hrs. Samples will be processed as in the preflight description.

Postflight

On R+3 and 8, 20.0 ml fasting blood samples will be collected for endocrine studies.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: Science objectives are the same as originally proposed. Inflight crew member participation has increased from two to four crew members. Requirements for fecal and urine samples have been deleted. Experiment protocol, design, and hardware requirements are essentially the same. The PI has gained experience with the dual, stable isotope method and original concerns over this technique have now been eliminated.

SCIENCE: Dr. Arnaud proposes to investigate the acute endocrine and calcium soft tissue changes that occur upon exposure to 0-g. The experiment seeks to determine the mechanisms responsible for the increased urinary and fecal losses and to define quantitatively changes in calcium intestinal absorption. The PI has extensive experience with the proposed endocrine measurements and uses the most sophisticated techniques available. Previous manned spaceflight missions have shown acute changes in urinary calcium excretion, and endocrine and intestinal changes are anticipated to occur within 7 days. Consequently, this category 3 proposal is consistent with the first announcement of opportunity evaluation criterion and meets the payload and program objectives.

The proposal involves the use of stable calcium isotopes to measure intestinal calcium absorption. At the time of categorization, development of these studies were not complete. The definition phase has resulted in validation of these studies and has shown that this important measurement is technically feasible.

EQUIPMENT: The PI plans to provide two kits, a tracer kit, and a -20°C stowage kit. LSLE required are the inflight blood collection system, the rack mounted centrifuge, refrigerator, and -20°C freezer. All of these items are planned LSLE items either under development or in the procurement cycle.

SUMMARY: This experiment elegantly addresses the important question of altered Ca absorption in weightlessness. Although there was early concern over the proposed use of stable calcium isotopes to examine gastrointestinal

Ca absorption, the definition phase has provided information which indicates this experiment can be successfully implemented on LS-1. The PI has extensive experience with the proposed endocrine measurements and employs the most sophisticated techniques. The proposal has a high likelihood of success and significant results are expected. It is recommended that this proposal tentatively be selected for flight on the understanding that specimens will be shared, four crew members will be studied aloft, some urine and fecal samples will be deleted, and the metabolic balance study will be somewhat modified.

FLOW CHART AND MEASUREMENT LIST FOR EXPERIMENT #305 - ARNAUD

TESTS	Preflight							Launch	Inflight					Recovery	Postflight	
	L -18	L -17	L -16	L -15	L -14	L -10	L -4		L +2	L +3	L +4	L +5	L +6		R +3	R +8
ENDOCRINE TEST: immunoreactive parathyroid hormone, immunoreactive calcitonin, ionized Vitamin D metabolites, ionized serum calcium, magnesium, phosphorus and protein	4					4	4		4			4			4	4
STABLE ISOTOPE PROTOCOL:																
INTESTINAL CALCIUM ABSORPTION (ratio of ^{48}Ca TO ^{46}Ca), TOTAL CALCIUM TURNOVER (dilution curve of ^{46}Ca), BONE RESORPTION (turnover - absorption)																
oral ingestion of ^{48}Ca isotope ($CaCO_3$)	4									4						
injection of ^{46}Ca isotope	4									4						
blood sample*	4**	4	4	4	4					4**	4	4	4			
DIET INFORMATION (calcium, phosphorus, protein)	Daily from L-48 to L-0								Daily from L+0 to R-0						Daily from R+0 to R+8	

* measurements will include ^{48}Ca/total calcium ratio and ^{46}Ca/total calcium ratio

** 3 blood collections: one prior to isotopes and two after isotopes

IMMUNOLOGY

PROPOSAL NUMBER: 781240

TITLE: Lymphocyte Proliferation in Weightlessness

SPECIMEN: Human

PRINCIPAL INVESTIGATOR: Cogoli A., Ph.D.
AFFILIATION: Laboratorium for Biochemie, Zurich, Switzerland

CoIs/AFFILIATIONS:
S. Criswell, Ph.D./University of Arizona

BACKGROUND: Dr. Cogoli plans to extend his current Spacelab 1 experiment to include new functional objectives which address lymphocyte proliferation and activation in various gravitational environments. The approach is to refly his SL-1 hardware and to add the use of a Life Sciences Laboratory Equipment (LSLE) variable-g centrifuge. The stimulation of lymphocytes will be assessed by their incorporation of tritiated thymidine into DNA. Protein synthesis will be measured by the incorporation of ^{14}C-leucine. Postflight analyses will be made of cell ultrastructure via electron microscopy.

Previous space flight results have been ambiguous since the effect of weightlessness could not be separated from the stress of recovery.

The addition of this experiment to LS-1 would provide information on human immune response in weightlessness.

PI OBJECTIVES: The primary objective of this investigation is to study the effect of weightlessness on lymphocyte activation in order to establish possible alterations of the cells responsible for the specific immune response during long duration spaceflight. Another objective is to gain further information on the mechanism of differentiation of mammalian cells in a "space environment" and to test the hypothesis outlined in the first hypothesis. The data collected should allow a prediction on the efficiency of the natural defense mechanism against infections in space.

PI HYPOTHESES: Low-g and weightlessness depress lymphocyte activity whereas high-g has a stimulatory effect. Cell shape might be influenced by gravity. A correlation between cell shape and growth has been found recently. Cytoplasmic streaming and consequently the biological clock of the cell might be influenced by gravity.

EXPERIMENT PLAN:

Preflight

On L-10 a 7.5 ml venous blood sample is collected from four crew members. For each crew member donor, three replicate cultures are prepared by mixing blood with culture medium (1:10). Concanavalin A, a mitogen, is added and each set of cultures are incubated for 3 days at different g-levels: low-g, 1-g, and 4-g.

After the 3 days, ^{14}C-leucine and ^{3}H-thymidine are injected into each culture and incubation is continued. After 5 hours, all cultures are removed,

injected with polyvinylpyrolidone (PVP) stored at -20°C, and analyzed postflight.

On L-1 another 7.5 ml blood sample is collected and the above protocol is repeated.

On L-0 four culture blocks, each containing four culture chambers, are filled with the culture medium and stowed at 4°C in the Spacelab. These will be used for the multi-g experiment. A 400 ml blood sample is taken from a healthy non-crew member donor. Lymphocytes are purified by the Ficoll/ Hypaque technique and eight identical samples of cell culture are prepared. Four samples are used for ground control experiments.

The second four samples are sealed in cell culture blocks, and delivered as a carry-on incubator. The cell cultures are maintained at 37°C by batteries until placed into the spacelab. Crew members are required on L-10 and L-1 for blood collection for approximately 10 minutes/session.

Inflight

Within 8 hours following launch, the experiment is activated by injection of mitogen into the culture chambers in the carry-on block case. No later than 20 hours post-launch, the block case is transferred to the Spacelab, rack mounted, and powered up. On Mission Days (MD) 2, 3, 4, and 5, ^{3}H-thymidine and ^{14}C-leucine are added to cultures 1-4, respectively, and incubation is continued for another 2 hours. At the end of each incubation, PVP is added to the culture and the culture is stowed at -20°C. The monitoring system is then turned off and stowed.

On MD 3, 10 ml of blood is collected from each of the four crew members participating in preflight experiments. Blood samples are defibrinated and allowed to clot. To the medium-filled culture chambers stored at -4°C in culture blocks preflight, 2.2 ml of defibrinated blood is added along with the mitogen. Four cell cultures are prepared for each crew member. Three replicates/crew are affixed to the variable-g centrifuge and the centrifuge switched on. The remaining samples are incubated at 37°C.

On MD 6, ^{14}C-leucine and ^{3}H-thymidine are injected into each of the 12 culture chambers and centrifugation, incubation is continued. Six hours after labeling, all culture chambers are removed, injected with PVP and stowed at -20°C.

Postflight

On R+1, 7, and 14, blood will be collected from each crew member and cultures set up as described for preflight L-10. On R+3, 10, and 17 the cultures will receive the tracer. After the specificed incubation period, all cultures will be frozen at -20°C.

On R+18, all samples (pre-, post-, inflight) will be shipped frozen to the PI for analysis and electron microscopy.

Ground Studies

There is one ground control study for this experiment. On L-0, flight and ground control experiments are prepared. The flight experiment is sealed in the block case at 37°C, while ground samples are incubated. On MD 2, 3, 4, and 5 the same inflight operations are performed with the ground control samples.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The PI initially proposed to use the SL-1 1NS101 Helianthus Flight Experiment (HEFLEX) centrifuge to conduct his study at gravitational environments of 0.3-g and 0.6-g. The hardware reflected in the Experiment Requirements Document (ERD) is a Life Sciences Laboratory Equipment (LSLE) variable-g centrifuge with 0.5-g, 1.0-g, and 4.0-g capability. A refrigerator and an incubator (37°C) were added as LSLE requirements in the ERD. Dr. Brown was deleted as CoI. The proposal asks for one or more crewmen and the ERD requests four crewmen. The overall experiment protocol was much more detailed in the ERD. One postflight experiment session was added in the ERD.

SCIENCE: This proposal by Dr. Cogoli will provide new information concerning the effects of spaceflight on the human immune system. In particular, this experiment will document any changes that occur in lymphocyte function and proliferation and will differentiate between changes due to operational stresses versus weightlessness per se. This is a relatively straightforward experiment asking fundamental questions, and will shed light on the system versus cellular response to weightlessness. This study is in direct agreement with the first two announcements of opportunity evaluation criteria as well as three of the four payload objectives, and is in direct accordance with the first two program objectives.

This ia a relatively simple and scientifically sound study requiring hardware currently under development for an SL-1 experiment. It is also an extension and improvement of previous Skylab studies. Therefore, it should have a high likelihood of success in space.

Definition activity on this proposal provided more detailed experiment protocols and included an extra postflight experiment session. Inclusion of this proposal on the LS-1 payload will provide a broad-based immunological study in an area of immune function in which changes have been previously observed.

EQUIPMENT: The PI supplied equipment includes cell culture blocks, a block case to maintain the cell cultures at 37°C, and a front panel assembly used to mount the block case in the Spacelab rack during Spacelab activation. The LSLE required are the inflight blood collection system, a refrigerator, and a freezer. Two items requested as LSLE, a Low-g centrifgue ($50K) and an incubator ($102K), are not planned LSLE at this time.

SUMMARY: This study addresses an area of immune function where changes have previously been observed. Operational medical data from STS-1 demonstrated a statistically significant decrease of the ability of lymphocytes to respond

to an *in vitro* mitogenic challenge postflight. Cogoli's experiment specifically addresses lymphocyte reactivity and will determine whether the weightlessness stress acts directly on the lymphocyte or whether it acts indirectly through the immune system. It is recommended that this investigation tentatively be selected for flight on the understanding that the PI will share specimens with other investigators.

FLOW CHART AND MEASUREMENT LIST FOR EXPERIMENT #240 - COGOLI

TESTS	Preflight						Launch	Inflight						Recovery	Postflight								
	-10	-9	-8	-7	-1	-0	+0	+1	+2	+3	+4	+5	+6	+1	+2	+3	+7	+8	+9	+10	+14	+15	+17
IN VITRO LYMPHOCYTE EXPOSURE TO MITOGEN (CONCANAVALIN A)																							
GRAVITATIONAL EFFECTS ON LYMPHOCYTE ACTIVATION*																							
Blood sample	4				4							4		4			4				4		
Sample preparation and addition of Mitogen	4				4							4		4			4				4		
Exposure to: 0.5, 1.0, and 4.0-G pre and postflight; 0.0, 0.5, 1.0, and 4.0-G inflight	4	4	4	4	4	4	4†	4†	4†			4	4	4	4	4	4	4	4	4	4	4	4
Addition of isotopes**				4					4†				4			4				4			4
Sample fixation				4					4†				4			4				4			4
KINETICS OF LYMPHOCYTE PROLIFERATION* ▲																							
Blood sample and lymphocyte purification (ficol/hypaque technique)						1																	
Addition of Mitogen							1																
Addition of isotopes**								1	1	1	1												
Sample fixation								1	1	1	1												

* Measurements will include:
DNA biosynthesis (^{3}H-thymidine incorporation into DNA)
Protein biosynthesis (^{14}C-leucine incorporation into protein)
Cell ultrastructure (electron microscopy)

** ^{3}H-thymidine and ^{14}C-leucine

▲ One subject with four inflight and four ground based syncronous samples

† Continuation of preflight control initiated on L-1 (to be performed on the ground)

GENERAL BIOLOGY

PROPOSAL NUMBER: 781236

TITLE: Determine Properties on the Gravitropic Response of Plants in the Absence of a Complicating G-Force

SPECIMEN: Oat Seedling

PRINCIPAL INVESTIGATOR: Brown, A. H., Ph.D.
AFFILIATION: University of Pennsylvania, Philadelphia, PA

CoIs/AFFILIATIONS:
A. Johnsson, Ph.D./University of Trondheim, Norway
D. K. Chapman, M.S./University of Pennsylvania, Philadelphia, PA

BACKGROUND: All developed plants exhibit a fundamentally similar growth pattern; i.e., roots orient themselves toward the ground while leaves and stems orient themselves away from the ground. It is clear that some gravity sensing mechanism exists, but the well-studied geotropic response mechanism remains a mystery. This experiment is part of a continuing series that will examine the geotropic response mechanism in Spacelab. In this study, the sensitivity or threshold level of the plant's gravity sensor to altered gravitational fields will be determined.

Growing plants respond to an altered gravitational field by vectored bending. On Earth, the inverse relationship between transverse g-stimulation (stimulus intensity) and stimulus duration required to achieve a given curvature is constant over a multifold range (Reciprocity Rule). By constructing dose-response relationships, a gravity sensing threshold may be determined.

PI OBJECTIVES: To determine the g-threshold exposure time of the gravity-sensing mechanism in a plant seedling. To test the validity of the reciprocity rule in a region impossible to be explored on earth.

PI HYPOTHESES: The gravitropic bending of a plant shoot will display the same kinetic features in weightlessness as at 1-g. The G x T product (intensity of transversely applied G force times duration of application) corresponding to a standardized response when measured in weightlessness will be constant over a wide range of G and T values. The constant obtained from data taken in weightlessness will be the same as that measured on earth.

EXPERIMENT PLAN:

Avena sativa (oat) seedlings will be used as the test organism. They will be planted on Earth and grown in the dark to an appropriate size, then placed onboard the shuttle.

Preflight

Several studies must be done prior to launch. A seed source will be selected. Seeds are purchased annually in batches and tested periodically for viability. The growth characteristics of the plant will be determined, particularly in regard to optimum temperature, medium and age. A geotropic response data base will be established in 1-g using flight-specific hardware for comparison to the hypotheses.

Within the last week prior to launch, 192 seeds are planted at intervals of 24 hours. Five age groups of seedlings are loaded into the PCOC (Plant Carry-On Container) and stowed in a middeck locker. Group 1 contains 96 plants to be used on the first day of the mission. Groups 2-5 each contain 24 plants to be used on the second through the fifth days.

Additional seeds are planted on Earth and loaded into a second PCOC so that if launch is delayed 24 hours or more the two PCOCs can be exchanged.

Inflight

One of the rotors is designated as a culture rotor; the other as a culture or experiment rotor. All plants are transferred to the rotors at first Spacelab access for growth at 1-g. Plants are transferred between culture and experiment rotors in order to administer a predetermined series of g-pulses to preselected groups of test plants.

It is expected that each rotor will hold 8 modules with 2 cubes of 6 plants each for a total sample size of 192 (2x8x2x6=192).

Twenty tests are planned (20 combinations of stimulus intensity and duration). Four tests are to be run per day. The plants are grown with their long axis parallel to an imposed 1-g field. For testing, the plants are oriented so that they experience the g-field transverse to their long axis. After the g-pulse, the rotor is slowed and time-lapse IR video images are stored and downlinked. After exposure, the plants are reoriented and stored on the 1-g rotor until fixation on the morning after exposure. IR video images are taken during this period of reorientation.

Postflight

The video tape will be analyzed in the PI's laboratory. Plant morphology will be studied. Additional studies will be performed as necessary to control for flight parameters.

Ground Control

No inflight ground control is necessary. Preflight data (collected using the flight hardware) will be used for comparison purposes and estimation of some of the experiment parameters. Postflight data collection will be used as necessary to compensate for flight anomalies.

Measurements

Type	Units
Relative humidity	%
Video tape **	N/A
Audio tape **	N/A
G-Threshold (GTHRES) status **	N/A
Video stills *	N/A
Bending response **	Degree
Acceleration**	g
Vibration**	g

Type	Units
Temperature**	°C
Carbon dioxide level**	% (by vol)
Ethylene level**	ppb

* Data taken during flight procedures only.
** Data taken during flight and ground procedures.
All other data taken on the ground.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: A change has been made in the number of plants per module. The original proposal called for 1 to 3 plants per module. In the Experiment Requirements Document (ERD), modules hold 2 cubes, each of which hold 6 plants for a total of 12 plants per module. This has increased the sample size.

Avena sativa L. was selected as the test specimen. Zea mays has problems with the growth of the mesocotyl. Avena has been used extensively in the past for geotropic studies.

A change has been made to fix the plants after stimulation. Plant morphology and anatomy will be examined postflight.

It was proposed originally to use both rotors to measure the G x T product at the same time. The investigator decided that all plants should be grown at 1-g so that straight coleoptiles result. In the ERD, one rotor has been designated as the experiment rotor and the other as the culture rotor. At the beginning of the flight experiment, both rotors operate at 1-g. Individual cubes on the experiment rotor will be reoriented to receive a transverse stimulus of 1-g for different durations, while the other plants on the rotor develop normally at 1-g. This continues until all of the cubes on the experiment rotor have been removed for specimen fixation. Then cubes from the culture rotor are transferred to the empty experiment rotor while the culture rotor continues to spin at 1-g. The experiment rotor provides gravity stimulations of less than 1-g on the culture rotor. Cubes from the culture rotor will be transferred to the experiment rotor for different G x T combinations until the supply is exhausted.

SCIENCE: This experiment is designed to improve the basic understanding of the mechanism by which a plant shoot senses gravity. It tests and extends the Reciprocity Rule by pulsing the plant at different g forces and observing the response uncomplicated by terrestrial 1-g. At different time intervals inflight, a transverse g vector will be applied and the resultant gravitropic bending will be monitored by time-lapse photography.

The experimental design, including supporting studies, is appropriate for this work. This is an extension of the clinostat and hypergravity work that Brown has been doing for years. It will extend the empirical evidence for the Reciprocity Rule to a region of g-values difficult to achieve on Earth.

If the variability among specimens given the same exposure is high, a larger sample size will be necessary. This will be achieved by limiting the range

of exposures (gravity magnitude and time) in flight; ultimately a reflight will be required to extend the range to the original value.

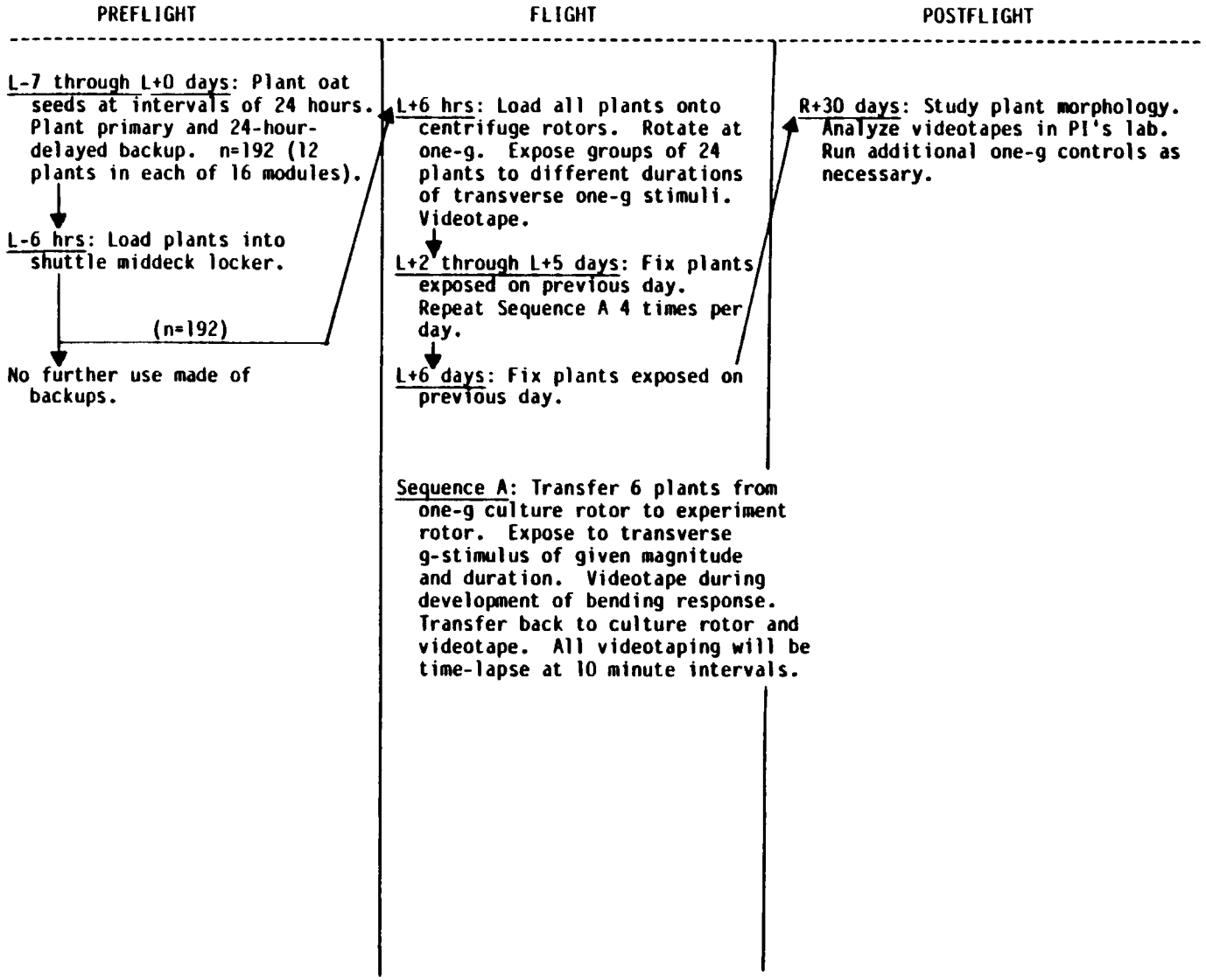
This experiment is responsive to AO evaluation criterion 2: to investigate significant biological phenomena that may occur during exposure to the space environment. It is of high merit, requires access to the space environment, and possesses a high likelihood of success. Therefore, it meets the payload objectives. Since it is designed to further knowledge of Earth biology, it also is consistent with the program objectives.

NASA has proposed that Heathcote use the hardware developed for this experiment to investigate the phototropic response of plants in zero-g (experiment 781054). By combining the two experiments in this way, the use of the hardware is optimized, and important information is gained about very basic responses of plants to weightlessness. In fact, the phototropic and geotropic responses of plants must be characterized before any substantial further work can be done.

EQUIPMENT: NASA should play a more active role in this experiment to ensure compliance with program R&QA requirements. The PI has had problems meeting NASA R&QA requirements for SL-1. His EMP includes extensive plans for redesign and refabrication of the existing HEFLEX. This represents a departure from his original plan to only make minor modifications to the HEFLEX hardware. The necessary experiment-unique equipment is the GTHRES hardware (a modification of HEFLEX), the plant carry-on container, a fixation kit, a container for the fixed plants, and a green safelight for the GPWS. LSLE to be used includes GPWS, RAU, and DEMS. Although Dr. Brown's machine shop built the HEFLEX hardware, a possibility exists that costs could be reduced by having the NASA shops perform the modifications to make the GTHRES hardware.

SUMMARY: This comprehensive proposal seeks to study the gravitropic response of plants to improve the basic understanding as to how a plant shoot senses gravity. (It would be complemented by Heathcote's proposal (781054) addressing a similar fundamental question). It has a high likelihood of success. Resource requirements are reasonable and the hardware development requirements should be minimal since only minor modifications to the existing HEFLEX hardware would be required. It is recommended that this proposal tentatively be selected for flight on the understanding that it share equipment with Heathcote and several procedural changes in the use of the HEFLEX device be accepted by the PI.

EXPERIMENT FLOW DIAGRAM - ES 236



PROPOSAL NUMBER: 781054

TITLE: Post Illumination Onset of Nutation at Zero Gravity

SPECIMEN: Wheat Plant

PRINCIPAL INVESTIGATOR: Heathcote, D. G., Ph.D.
AFFILIATION: University College, Cardiff, Wales, U.K.

CoIs/AFFILIATIONS:
A. H. Brown, Ph.D./University of Pennsylvania, Philadelphia, PA

BACKGROUND: Over evolutionary time, green plants have evolved complex attitude control systems which work to ensure that the various plant organs - roots, shoots, leaves, etc. - take up positions appropriate to their functions. Thus roots normally grow downward in the direction in which supplies of water and dissolved mineral salts are most likely to be found, and shoots grow upwards towards the aerial environment where leaves can be expanded to undertake the light-harvesting and gaseous exchange processes required for photosynthesis. The control system acts to correct any naturally occurring or experimentally produced deviations from the normal growth direction by modulating growth rates on opposite flanks of the organ to bring about a corrective bending. The main environmental cues which are used by plants as references are the direction of the gravity vector and the direction of incident light. The response induced by changes in the position of the plant with respect to the gravity vector is termed 'geotropic'; 'phototropic' responses result from changes in respect to light direction. In nature these processes interact and, together with other more minor controls, determine the attitude in space of the plant organ. It is relatively easy to study the geotropic response in isolation from the phototropic control, since experiments can be undertaken in the dark or in light of wavelengths which are not phototropically active. Pure phototropic behaviour cannot, however, be observed in the earth's gravitational field. Any phototropic movement induced by lateral light stimulation inevitably changes the position of the organ with respect to the gravity vector, and thus entrains a response from the geotropic mechanism. This situation parallels that of mammalian balance where both gravitational (otolith) and visual cues are used. Visual stimuli can be excluded by blindfolding, but otolith stimulation is more difficult to eradicate.

PI OBJECTIVES: To determine the time course of plant seedling curvature induced by phototropic stimulation in a microgravity environment.

PI HYPOTHESES: Seedling curvature proceeds at the same rate and direction in microgravity as in unit gravity. The extent of seedling curvature is the same in microgravity as in unit gravity. The seedling curvature does not exhibit reversal or lead to oscillations. The phototropic dose-response relationship is the same in microgravity as in unit gravity.

EXPERIMENT PLAN:

Triticum aestivum (wheat) will be used as the test organism. Seeds will be planted on Earth, and the seedlings will be grown inflight in the dark.

Preflight

Several studies must be done prior to launch. A seed source must be selected. Seeds will be purchased annually in batches and tested periodically for viability. The growth characteristics of the plant must be determined, particularly in regard to optimum temperature, growth medium, and age. The phototropic dose/response curve must be determined in order to guide the flight experiment. Baseline studies will be performed. Within the last few days before launch, a total of 96 seeds are planted in modules at intervals of 8 hours. Four groups of seeds are planted. Each age group is color-coded and consists of 4 modules of 6 plants per module. The 16 modules are packed into the PCOC (Plant Carry-On Container), carried onboard at late access and stowed in a middeck locker. Additional seeds are planted on a delayed timeline and placed in a second PCOC so that if launch is delayed more than 24 hours, the spacecraft can be entered and the two PCOCs can be exchanged.

Inflight

The experiment begins by growing the seedlings at 1-g on the centrifuge. When the appropriate age is reached, 4 modules at a time are transferred from the centrifuge to the recording and stimulus chamber (REST). Within the chamber, plants are monitored with IR video in time-lapse mode at 10 minute intervals. After 5 hours of baseline measurements, the plants are stimulated phototropically with a lateral light source for a brief period of time (each module can have a different stimulation). Time-lapse IR monitoring then continues for 3 hours for a short run or 11 hours for a long run. At the end of the run, the plants are fixed and stowed, and a new run is started.

Postflight

The video tape will be analyzed in detail. Plant anatomy will be studied. Additional 1-g controls will be run as necessary to compensate for flight parameters.

Ground Control

No inflight ground control is necessary. Preflight data (collected using the flight hardware) will be used for comparison purposes and estimation of some of the experiment parameters. Postflight data collection will be used as necessary to compensate for flight anomalies.

Measurements

Type	Units
Relative humidity	%
Video tape **	N/A
Audio tape **	N/A
Video stills *	N/A
FORTRAN status **	N/A
Bending response **	Deg/min
Acceleration (0-20Hz)*	g
Vibration (20-2000Hz)**	g
Temperature*	°C

Type	Units
CO_2 content of air*	ppm
C_2H_4 content of air*	ppb

* Data taken during flight procedures only.
** Data taken during flight and ground procedures.
All other data taken on the ground.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The scope of the experiment has been increased to include phototropic dose response curves. Plants in each module will be stimulated by a different duration of light, and the bending of plants will be followed as before with an IR video camera. The phototropic dose response curves will be compared with those generated on earth.

The test specimen has been changed to Triticum aestivum coleoptiles from Phaseolus aureus seedlings. The curvature of Triticum coleoptiles can be easily measured. Triticum produces a very small mesocotyl when grown in the dark.

Two rotors with 16 modules total are planned in the Experiment Requirements Document (ERD). Each module will hold 6 plants. This will increase the sample size.

Two types of experiment runs are planned in the ERD. In a short run, plant modules will be transferred from the rotor to 0-g where they are photographed for 5 hours while circumnutation damps out. These plants are stimulated phototropically and photographed for an additional 3 hours. This run is used for stimulus doses that are expected to yield small responses. The total duration of the short run is 8 hours. In a long run, plants are transferred from the rotor to 0-g and photographed for 5 hours as in the short run. They are then stimulated phototropically and photographed for 11 additional hours. This run is used for doses expected to produce maximum curvatures. This allows any subsequent induced oscillations or autotropic responses to be followed. The total duration of the long run is 16 hours. Two short runs and two long runs are planned.

A change has been made to preserve the plants after they have been exposed. Plant morphology and anatomy will be examined postflight.

SCIENCE: The experiment addresses a question of basic plant gravitational biology. The experimental design (including all supporting studies) is appropriate for testing the hypotheses. This is a excellent experiment in that regardless of the outcome (barring hardware failure), the results will answer basic questions about plant phototropic responses. The investigator proposes to determine basic phototropic responses of wheat plants in the absence of gravity. This type of experiment is fundamental to gravitational biology and offers the NASA an opportunity to provide basic science research in the spacelab environment.

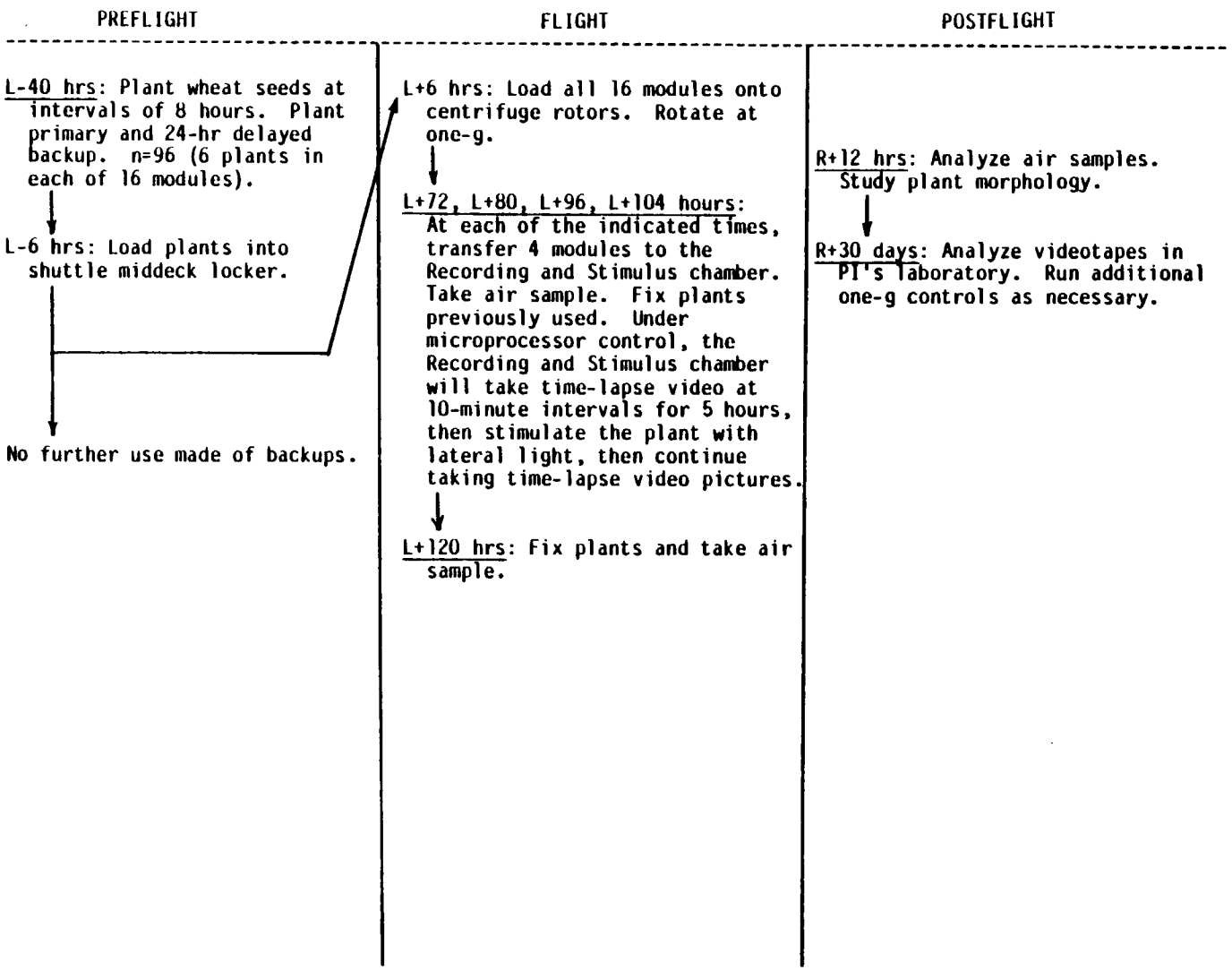
As a result of the definition phase activities, it was determined that this investigation could easily be dovetailed with another plant experiment which requires similar hardware (Heflex plant unit) and support (781236, Brown). It is assumed that if the investigation is selected, then this investigator would agree to the provision that this experiment and that of Brown be combined into the necessary flight hardware. Informal discusssions with this investigator and Brown have indicated that this would pose no problem.

The definition phase activity has demonstrated that, if this experiment shares the flight hardware with that of Brown, it should be considered to have the status of a strong proposal.

EQUIPMENT: NASA should play a more active role in this experiment to ensure compliance with program R&QA requirements. The success of this experiment is dependent on the support of the Brown who would supply the major hardware items. Experiment unique equipment for the experiment include FORTRAN hardware; plant carry-on container; fixation kit: fixed plant container; and an air sampling kit. The LSLE required for this experiment includes: GPWS; DEMS; and the RAU.

SUMMARY: This is a well designed experiment which would strongly complement the experiment proposed by Brown (781236). Both experiments examine fundamental tropisms: Brown studying gravity and Heathcote studying light. The investigator is well published in his field. The concept of sharing flight hardware and much of the support hardware makes it very attractive to include this experiment in the dedicated spacelab. It is recommended that this investigation tentatively be selected for flight in the understanding that it share equipment with Brown and that several procedural changes in the use of the HEFLEX device be accepted by the PI.

EXPERIMENT FLOW DIAGRAM - ES 054



PROPOSAL NUMBER: 781256

TITLE: The Effect of Weightlessness on the Development of Amphibian Eggs Fertilized in Space

SPECIMEN: Frog Eggs

PRINCIPAL INVESTIGATOR: *Tremor, J. W., Ph.D.
AFFILIATION: NASA-ARC, Moffett Field, CA

CoIs/AFFILIATIONS:
*K. A. Souza/NASA-ARC, Moffett Field, CA
G. W. Nace, Ph.D./University of Michigan, Ann Arbor, MI
M. D. Ross, Ph.D./University of Michigan, Ann Arbor, MI

BACKGROUND: Whether or not gravity plays an important role in early development of amphibian eggs has been a long debated issue. Studies with hypergravity in a centrifuge and studies with mechanical re-orientation of eggs, seem to have led only to controversy. Also, frog eggs fertilized on Earth have been flown on Biosatellite II with no obvious influence on development; however, the most sensitive periods may pertain to the events that occur during and shortly after fertilization. It is at this time that the egg rotates due to a polarized distribution of heavier yolk material and that bilateral symmetry becomes evident even before the first cleavage.

PI OBJECTIVES: To determine the role of gravity on the processes of fertilization, bilateral symmetry determination, and early development in amphibians.

To determine the influence of microgravity exposure during fertilization and early development on the subsequent development at 1-g through metamorphosis and subsequent inbred generations.

To examine, in particular, the development of the inner ear structure and associated gravity sensitive components for abnormalities.

PI HYPOTHESES: 0-g exposure is expected to significantly alter the processes of (1) fertilization, (2) bilateral symmetry determination, and (3) early development of frog eggs.

After a 10 ± 3 day exposure to 0-g and the return to 1-g conditions, the subsequent developmental processes will be significantly different from normal at 1-g only if continued through metamorphosis and subsequent inbred generations.

The inner ear structures, such as the otolith and lime sacs will be significantly altered from normal after the 0-g exposure and subsequent development.

*Dr. Tremor requested K. Souza replace him as P.I. on this study.

EXPERIMENT PLAN:

Preflight

From a population of 100 female and 50 male frogs maintained at 4°C at KSC, 20 females will be selected and induced to ovulate by injections of pituitary extract. Frog sperm solutions will be prepared at L-8 hours. Six females will be selected for flight.

Inflight

The experiment will be conducted at a controlled temperature of 18°C. Fertilization tests at both 0-g and on a 1-g centrifuge will be conducted first with each of the 6 frogs. From 10 to 20 eggs are stripped into each of two egg chambers for each frog, washed with sperm solution, and placed at 1-g or 0-g. The subsequent experiment will involve three selected donor females and depend in part on the outcome of fertilization tests. For each female, 10 eggs will be stripped into each of 20 egg chambers and washed with sperm solution. Half will be placed on the 1-g centrifuge and half at 0-g for fertilization and subsequent development. If only 1-g fertilization is possible, all 20 chambers per frog will be placed at 1-g first and then half allowed to develop at 0-g. Two 1-g chambers and two 0-g chambers per frog will be fixed at intervals of 2-1/2, 24, and 58 hours after fertilization and, as practical, at a time prior to reentry.

Postflight

In addition to the examination of the fixed embryo stage, the remaining live embryos (two egg chambers for each of the three frogs) will be allowed to develop for study of later stages and for possible effects on subsequent generations.

Ground Control

Ground controls will involve testing fertilization success of the 14 remaining female frogs synchronized in time to the flight protocol.

Measurements

Type	Units
Frog maintenance temperature 4°C**	°C
Frog maintenance temperature 28°C**	°C
Egg chamber centrifuge temperature**	°C
Egg chamber incubator temperature**	°C
Egg chamber centrifuge revolutions**	RPM
Egg maturation microscopy	N/A
Fertilization success	%
Fertilized eggs microscopy	N/A
2 hour development stage microscopy	N/A
24 hour development stage microscopy	N/A
58 hour development stage microscopy	N/A
Reentry development stage microscopy	N/A
Postflight development	N/A

Type	Units
Otolith and lime sacs specific development	N/A
Postflight reproduction success	N/A

* Data taken during flight procedures only.
** Data taken during flight and ground procedures.
All other data taken on the ground.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: There are no substantive differences between the proposal and the Experiment Requirements Document (ERD) for this experiment. The ERD represents an improvement in specific experimental design. Fewer developmental stages are now being selected, but not to the detriment of the basic issues addressed in the proposal. The option to load prepared sperm solutions at last possible access, rather than prepare these solutions on orbit from intact testes or through disections of male frogs, was indicated in the ERD.

SCIENCE: This study addresses a well known historic topic in developmental biology and clearly requires the experimental condition of zero-g offered by spaceflight. The suggested experimental design with one-g inflight control should yield unequivocal results to assess requirements of a gravitational field at the time of fertilization and symmetry determination for subsequent normal development in the amphibian species selected. The study complements and should extend the knowledge gained from related studies on Biosatellite II.

Originally classified in category 1, the results of Definition Phase strengthen the support of this proposal as a potential flight experiment. This proposal is responsive to the second and third AO evaluation criteria. It is a well-designed study and, because of the prior experience of the investigator, is assured of a high likelihood of success. It therefore meets the payload objectives, as well as the program objectives.

The experiment as proposed is demanding of crew time early after Spacelab activation. The viability of sperm solutions and egg quality is time-critical. In order to fit the experiment into the payload, NASA has proposed that certain modifications be made to allow the experiment to be started on the third day of flight. Dr. Tremor has agreed in principle, but additional studies should be completed.

EQUIPMENT: The majority of the hardware required for development is straightforward.

The experiment-unique equipment is extensive because this is the only experiment to use frogs:

- Frog holding units (6)
- Frog egg chambers (54)
- Fixative vials (6)
- Sperm solution holder
- Sperm transporter (4°C)
- Spring water holder

1-G centrifuge (18°C)
Frog transporter (18°C)
Data recorder
Fixative holder
Stowage chamber (18°C)
Kits (fixation, frog-handling)

Maximum use is made of LSLE:

GPWS
DEMS
Dissecting microscope

SUMMARY: This study addresses a fundamental issue in developmental biology: does gravity have an effect on the symmetry of the developing amphibian embryo? The experimental design is well-conceived and the use of resources is modest. It has a high likelihood of attaining significant scientific results. It is recommended that this proposal tentatively be selected for flight on the understanding that the sperm solutions be prepared on the ground.

EXPERIMENT FLOW DIAGRAM - ES 256

PREFLIGHT	INFLIGHT	POSTFLIGHT
At KSC, 100 female and 50 male frogs maintained at 4°C. Inject 20 females with pituitary extract at L-24 hours and place at 18°C. Select six females for flight and six ground controls and load with equipment at L-12 to 14 hours. Maintain females at 18°C at all times. Prepare 4°C sperm suspension at L-8 hours and maintain at 4°C in sperm transporter. Load 4°C sperm suspension in transporter into space vehicle as convenient.	At L+6 hours remove sperm solution from 4°C to 18°C. At L+7 hours strip 10-20 eggs from each of six frogs into each of 2 chambers and into one fixative vial which is then fixed. Fertilize others and place one chamber at 1-g centrifuge and the other chamber at 0-g for ten minutes. Wash eggs and place both on centrifuge at 1-g for 30-40 minutes. Inspect for fertilization success and to select the donor females for experiment. At L+8.5 hours select 3 females. Strip 10 eggs into each of 20 chambers for each frog. Fertilize and place 1/2 of chambers per frog on 1-g centrifuge for 10 minutes. Wash all 20 chambers for each frog and replace the 1-g controls on centrifuge. Proceed sequentially/frog. Fix two chambers from 1-g and from 0-g each for each frog at intervals: 2 1/2 hours after fertilization 24 hours after fertilization 58 hours after fertilization Before re-entry as is practical	Continue maintenance at 18°C for remaining tadpoles and return to PI for continued study along with fixed materials.

PROPOSAL NUMBER: 781194

TITLE: Bone, Calcium, and Spaceflight

SPECIMEN: Rat

PRINCIPAL INVESTIGATOR: Holton, E. M., Ph.D.
AFFILIATION: NASA-ARC, Moffett Field, CA

CoIs/AFFILIATIONS:
C. E. Cann, Ph.D./University of California Medical Center, San Francisco, CA

BACKGROUND: Densitometric scans of heel bones in Skylab astronauts demonstrated a decrease in bone density. Experiments with rats on Cosmos 782 determined a mineral loss and also indicated an inhibition of periosteal bone formation in the tibia. Further rat experiments during Cosmos 936 led to the conclusions that, in rats, there was (1) no significant change in pore size, (2) a significant decrease in torsional strength, and (3) a reduction in periosteal bone formation. However, all significant differences disappeared within twenty-five days postflight.

PI OBJECTIVES: To determine the length of flight time required to significantly inhibit bone formation in both juvenile and adult male rats. To determine inflight total skeletal formation and resorption, as well as calcium absorption and excretion.

PI HYPOTHESES: Bone formation ceases sometime during the first 2-3 weeks of a spaceflight. Cessation is gradual, not abrupt. Negative calcium balance can be caused by a change in bone formation relative to bone resorption or by a change in calcium excretion relative to calcium absorption.

EXPERIMENT PLAN:

Preflight

Forty male rats, 20 rapidly growing juveniles and 20 slowly growing adults, will be preconditioned in habituation cages for 1 to 2 weeks prior to launch. Feces and dry urine collections will be made from each cage every other day to be analyzed for calcium. The diet for each animal will be the standard Research Animal Holding Facility (RAHF) flight diet until 24 hours before launch (L-1). At this time, a diet containing the stable isotope ^{40}Ca will be substituted and continued throughout the flight experiment. Tetracycline (10mg/Kg-intraperitoneally) will also be administered to each animal at this time.

Inflight

During the flight all of the animals will be maintained within the RAHF. The rats will receive the ^{40}Ca isotope through the food bar. The crew will be required to collect the waste trays from each cage on a daily basis. On the next to last flight day (L-6) each animal will receive an injection of a fluorescent tetracycline. An alternative plan would be to delay this injection until after reentry and perform it at KSC postflight.

Postflight

As soon as possible after recovery of the animals from Spacelab, one half of the group will receive an injection of a tritiated protein to determine osteoblast and osteoclast activity. These animals will be sacrificed within 24 hours of return (R+1). The remaining animals will be sacrificed 7 and 27 days postflight. Osteoblast and osteoclast activity will be determined by autoradiography. Bone morphology will be determined by computer aided fluorescent microscopy. The calcium turnover rate will be determined by neutron activation analysis.

Ground Controls

A group of animals (approximately 10) will be sacrificed preflight to serve as a baseline.

Another group of animals, equal in number to the flight group (40), will be used as a delayed synchronous ground control. These animals will experience the same experimental protocol as the flight group except for the spaceflight environment. The postflight sacrifice schedule will be identical to that of the flight group.

Measurements

Type	Units
Collection of feces and dried urine**	
Food consumption**	gm/day
Water consumption**	ml/day
Activity*	counts/day
Time of waste collection*	Days, hours, minutes
Morphology of tibia, humerus, femur, vertebrae	N/A
Osteoblast and osteoclast activity (autoradiography)	counts/min
Urine and feces calcium	mEq/l,mEq/g
Bone calcium	mEq/g
Bone length	mm
Bone width § Computer aided fluorescence	mm
Bone growth § microscopy	mm/day
Calcium turnover rate	ug/min/mg
Calcium content of rib muscle	mEq/g
Calcium content of blood	mEq/l
Temperature*	°C

* Data taken during flight procedures only.
** Data taken during flight and ground procedures.
All other data taken on the ground.

EXPERIMENT FLOW CHART: See attached.

DEFINITION ACTIVITY: The major differences between the proposal submitted and the Experiment Requirements Document (ERD) are the methods used to

determine calcium turnover and bone morphology. The original plan was to incorporate a tetracycline derivative in the rat food. This, combined with an injection of a different derivative pre- and postflight, provided fluorescent markers to determine the rate of growth in the bone. The PI has since developed and tested a new technique using a computer-aided fluorescence microscope which obviates the need for the inflight delivery of the tetracycline.

For calcium turnover studies, the initial proposal called for an injection of ^{47}Ca midflight and the administration of chromic oxide in the food as a fecal marker. Again, the PI has developed and tested a new technique which eliminates the need for the administration of these compounds. A stable isotope of calcium (^{40}Ca) will be compounded into the rat food in place of the standard calcium phosphate. The stable isotope is incorporated into the bone and the turnover rate is determined postflight by neutron activation analysis.

SCIENCE: This is a well planned experiment, firmly supported by previous Cosmos flight data. It combines two experiments, calcium turnover and bone growth, into one impressive package. The design is excellent in its approach to the logistics of flight and its attempts to utilize ground-based models in the interpretation of the data. The analytical methods have been proven on Cosmos flights.

This area of research addresses an important observation in spaceflight and as such is responsive to the objectives of the program. Changes in bone metabolism are important to the long term objectives of the NASA.

During the definition phase, some of the questions about the use of rats in the determination of bone changes in weightlessness were addressed. The investigator utilizes the rat bone model as an indication of the effects of spaceflight on bone systems (rats) and the mechanism of such changes. The inclusion of the bone labelling and tracer studies adds much to the investigation as it will provide information about the sequences and timing involved in the changes in bone.

Because of the nature of the experiment, and the ability of the investigation to utilize flight animals from other experiments, this is an excellent experiment to consider for animal sharing. Some ground-based data must be collected as to the interaction of the labelling technique with other experiments.

Definition phase activities provided even more confidence in the fact that this experiment is in a good state of readiness. The P.I. has provided good supporting studies and has continued to demonstrate an outstanding capability to support flight experiments and the LSFEP. As a result of the definition phase activity the P.I. has demonstrated that she could utilize as few as 5 flight animals and still get significant data.

EQUIPMENT: The PI's only hardware requirement is for two storage containers for RAHF waste trays. The experiment unique equipment required for this experiment is as follows: injection kit; food mixer; plastic bags; waste tray stowage containers; and modified waste trays. LSLE required for this experiment are: RAHF and GPWS.

SUMMARY: This investigation addresses both calcium turnover and bone growth. Analytical methods have been verified on Cosmos and ground-based experiments using hypokinetic models. It is recommended that this proposal tentatively be selected for flight on the understanding that animals be shared, inflight administration of tetracycline be eliminated, and the sample size be somewhat reduced.

EXPERIMENT FLOW DIAGRAM - ES 194